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(71) Applicant: WYETH [US/US]; Five Giralda Farms, Madison, NJ 07940 (US).

(72) Inventors: TREPICCHIO, William, L.; 21 Abbot Bridge Drive, Andover, MA 01810 (US). OESTREICHER, Judith, L.; 125 Main Street, No. 62, Newmarket, NH 03857 (US). LEONARD, John, P.; 89 Tennyson Drive, Manchester, NH 03104 (US). DORNER, Andrew, J.; 20 Baskin Road, Lexington, MA 02173 (US). HUNTER, Sharon, E.; 28 9th Avenue, Haverhill, MA 01830 (US).

(74) Agents: MANDRA, Raymond, R. et al.; Fitzpatrick, Cella, Harper & Scinto, 30 Rockefeller Plaza, New York, NY 10112-3801 (US).

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(54) Title: METHODS FOR DIAGNOSING AND TREATING MULTIPLE SCLEROSIS AND COMPOSITIONS THEREOF

(57) Abstract: The present invention is directed to novel methods for diagnosis and prognosis of Multiple Sclerosis by identifying differentially expressed genes. Moreover, the present invention is also directed to methods that can be used to screen test compounds and therapies for the ability to inhibit multiple sclerosis. Additionally, methods and molecule targets (genes and their products) for therapeutic intervention in multiple sclerosis are described.

- 1 -

TITLE

**METHODS FOR DIAGNOSING AND TREATING
MULTIPLE SCLEROSIS AND COMPOSITIONS THEREOF**

5

[0001] This application claims benefit of U.S.
Provisional Patent Application No. 60/280,572, filed
March 30, 2001.

10   Field of the Invention

[0002] The present invention is directed to novel
methods for diagnosis and prognosis of Multiple
Sclerosis by identifying differentially expressed

15   genes. Additionally, methods and molecular targets
(genes and their products) for therapeutic intervention
in multiple sclerosis are described.

Background of the Invention

20

[0003] Multiple Sclerosis (MS) is a chronic, often
disabling autoimmune disease of the central nervous
system that first appears in young adults, with greater
occurrence in women than men. MS involves an

25   inflammation of the central nervous system, in which
lymphocytes attack myelin and oligodendrocytes to leave
behind characteristic lesions or plaques in the brain
and spinal cord. The involvement of complex

- 2 -

immunological pathways, in particular systemic T-cell
mediated pathways, has presented a challenge to
researchers attempting to elucidate the mechanism of
the disease.

5

[0003] Population studies on MS have suggested that MS
is a product of genetic susceptibility as compounded by
environmental exposure of an unknown nature. Genetic
susceptibility of the disease has been borne out by a
10   correlated familial risk, showing the greatest
recurrence among monozygotic twins, then among full-
siblings and then cousins. Dyment et al. "Genetics of
Multiple Sclerosis" Human Molecular Genetics, 6: 1693-
1698 (1997). A connection to the environment has
15   further been drawn by several-fold differences in risk
between populations in different latitudes, with
increasing recurrence in higher latitudes with cooler
climates (i.e. northern Europe or Canada). Dyment et
al. Population studies however, have been unable to
20   clearly identify all of the genes involved in MS and
their complex interaction, nor have these studies been
able to provide significant guidance on treatment or
therapy for MS.

25   [0004] The etiology of MS remains unclear, although
numerous T-helper cell inflammatory pathways have been
implicated in MS pathology. Due to the complex
interaction of many genes expressed in these cell-
mediated immune pathways, current methodologies have
30   yielded limited success in elucidating the disease.
Various methods such as differential display, in situ
hybridization, reverse transcriptase-polymerase chain
reaction or competitive polymerase chain reaction have
been used to study the patterns of gene expression with
35   limited success. Baranzini et al. "Transcriptional

- 3 -

Analysis of Multiple Sclerosis Brain Lesions Reveals a
Complex Pattern of Cytokine Expression," *J. Immunol.*
165: 6576-6582 (2000); van Boxel-Dezaire et al.
"Decreased Interleukin-10 and Increased Interleukin-

5   12p40 mRNA are Associated with Disease Activity and
Characterize Different Disease Stages in Multiple
Sclerosis" *Annals of Neurology*, 45: 695-703 (1999)). A
study by Whitney and colleagues utilized genetic
expression data from one patient to compare MS tissue

10 to non-MS tissue, revealing that about sixty-two genes
were differentially expressed, including the Duffy
chemokine receptor, interferon regulatory factor-2, and
tumor necrosis factor alpha receptor-2. Nonetheless,
no method has yet been developed to provide

15 comprehensive data on differentially expressed genes
from multiple subjects at multiple stages of
development, or through a wide range of geographical
regions.

20 [0005]   Furthermore, the nature and variability of MS
as expressed in different individuals has proven to be
a challenge in characterizing the disease and in
providing a prognosis for each patient. Three general
stages or types of MS have been characterized: (1)

25 secondary progressive, in which symptoms and disability
gradually worsen over time as opposed to having
discreet recognizable attacks or relapses; (2) primary
progressive, in which there are no attacks but a
gradual worsening from the start; and (3) relapsing-

30 remitting, in which there is a clinical worsening of
symptoms followed temporary improvement. The present
invention therefore addresses these issues by using
differentially expressed genes to provide methods for
diagnosis and prognosis, and assays for therapeutic

35 intervention.

- 4 -

SUMMARY OF THE INVENTION

[0006]   In one embodiment, the invention provides a
method of diagnosing a subject with multiple sclerosis
by comparing the level of expression of a marker in a
sample from a subject, where the marker is selected
from the group of markers set forth in Tables 1-5 (for
the animal model of multiple sclerosis) or 7-10, to the
normal level of expression of the marker in a control
sample, where a substantial difference between the
level of expression of the marker in the sample from
the subject and the normal level is an indication that
the subject is afflicted with multiple sclerosis. In a
preferred embodiment, the marker corresponds to a
transcribed polynucleotide or a portion thereof.
Preferably, the marker corresponds to a transcribed
polynucleotide or a portion thereof, and the sample is
collected from brain tissue or comprises peripheral
blood mononuclear cells (PBMCs). In another preferred
embodiment, the control sample is from non-involved
tissue from the subject. Alternatively, the control
sample is from the tissue of a nondiseased subject. In
a further preferred embodiment, the level of expression
of the marker in the sample differs from the normal
level of expression of the marker in a subject not
afflicted by a factor of at least two, and in an even
more preferred embodiment, the expression levels differ
by a factor of at least five.

[0007]   In another preferred embodiment, the level of
expression of the marker in the sample is assessed by
detecting the presence in the sample of a protein
corresponding to the marker. In a particularly
preferred embodiment, the presence of the protein is

detected using a reagent which specifically binds with the protein. In an even more preferred embodiment, the reagent comprises an antibody or fragments thereof. In another preferred embodiment, the method comprises a

5   marker selected from markers listed in Table 9 or 10. In another preferred embodiment, the level of expression of the marker in the sample is assessed by detecting the presence in the sample of a transcribed polynucleotide or portion thereof, where the

10   transcribed polynucleotide includes the marker. In a particularly preferred embodiment, the transcribed polynucleotide is an mRNA or a cDNA.

[0008]   In yet another preferred embodiment, the level

15   of expression of the marker in the sample is assessed by detecting the presence in the sample of a transcribed polynucleotide or a portion thereof which hybridizes with a labeled probe under stringent conditions, wherein the transcribed polynucleotide comprises the marker

20

[0009]   In another preferred embodiment for diagnosing a subject with multiple sclerosis, the level of expression in the sample of each of a panel of markers independently selected from the markers listed in

25   Tables 1-5 or 7-10 is compared with the normal level of expression of the same panel of markers in a control sample, where the level of expression of more than one of the markers is substantially different, relative to the corresponding normal levels of expression of the

30   markers, indicating that the subject is afflicted with multiple sclerosis. In a particularly preferred embodiment, the plurality includes at least five of the markers set forth in Tables 1-5 or 7-10.

- 6 -

[0010]   In another embodiment, the invention provides a
method of monitoring the progression of multiple
sclerosis in a subject, including detecting in a
subject sample at a first point in time the expression
5   of marker, where the marker is selected from the group
including the markers listed in Tables 1-5 or 7-10,
repeating this detection step at a subsequent point in
time with the same marker, and detecting a substantial
difference between the levels of expression, thus
10   indicating that the subject has progressed to a
different stage of multiple sclerosis. In a preferred
embodiment, at least 5 markers are selected from the
group of markers Tables 1-5 (murine) or 7-10 (human)
and combinations thereof. In another preferred
15   embodiment, the marker corresponds to a transcribed
polynucleotide or portion thereof, where the
polynucleotide includes the marker. In a particularly
preferred embodiment, the cells are collected from
brain or blood tissue (PBMCs).
20

[0011]   In another embodiment, the invention provides a
method of assessing the efficacy of a test compound for
inhibiting multiple sclerosis in a subject, including
comparing expression of a marker in a first sample
25   obtained from the subject which is exposed to or
maintained in the presence of the test compound, where
the marker is selected from the group including the
markers listed in Tables 1-5 (murine) or 7-10 (human),
to expression of the marker in a second sample obtained
30   from the subject, where the second sample is not
exposed to the test compound, where a substantially
different level of expression of the marker in the
first sample relative to that in the second sample is
an indication that the test compound is efficacious for
35   inhibiting multiple sclerosis in the subject. In a

- 7 -

preferred embodiment, the first and second samples are
portions of a single sample obtained from the subject.
In a particularly preferred embodiment, the
substantially different level of expression is a lower
5   level of expression in the first sample.

[0012]   In another embodiment, the invention provides a
method of assessing the efficacy of a therapy for
inhibiting multiple sclerosis in a subject, the method
10 including comparing expression of a marker in the first
sample obtained from the subject prior to providing at
least a portion of the therapy to the subject, where
the marker is selected from the group including the
markers listed in Tables 1-5 (murine) or 7-10 (human),
15 to expression of the marker in a second sample obtained
from the subject following provision of the portion of
the therapy, where a substantially different level of
expression of the marker in the second sample relative
to the first sample, is an indication that the therapy
20 is efficacious for inhibiting multiple sclerosis in the
subject.   In a preferred embodiment, the substantially
different level of expression is a substantially lower
level of expression in the second sample.   In a
particularly preferred embodiment, the method further
25 comprises a step of comparing expression of the marker
in a control sample, where a substantially similar
level of expression in the second sample, relative to
the control sample, is an additional indication that
the test compound is efficacious for inhibiting
30 multiple sclerosis.

[0013]   In another embodiment, the invention provides a
method of screening test compounds for inhibitors of
multiple sclerosis in a subject, the method including
35 obtaining a sample including cells from a subject,

- 8 -

separately maintaining aliquots of the sample in the
presence of a plurality of test compounds, comparing
expression of a marker in each of the aliquots, where
the marker is selected from the group including the
5   markers listed in Tables 1-5 (murine) or 7-10 (human),
and selecting one of the test compounds which induces a
substantially different level of expression of the
marker in the aliquot containing that test compound,
relative to other test compounds. In a particularly
10   preferred embodiment, the substantially different level
of expression is a substantially lower level of
expression. In an alternative preferred embodiment,
the substantially different level of expression is a
substantially enhanced level of expression.

15

[0014]   In another embodiment, the invention provides a
kit for diagnosing a subject with multiple sclerosis,
including reagents for assessing expression of a marker
selected from the group including the markers listed in
20   Tables 1-5 (murine) or 7-10 (human).

[0015]   In another embodiment, the invention provides a
kit for diagnosing multiple sclerosis in a subject, the
kit including a nucleic acid probe where the probe
25   specifically binds with a transcribed polynucleotide
corresponding to a marker selected from the group
including the markers listed in Tables 1-5 (murine) or
7-10 (human).

30   [0016]   In another embodiment, the invention provides a
kit for assessing the suitability of each of a
plurality of compounds for inhibiting multiple
sclerosis, the kit including a plurality of compounds
and a reagent for assessing expression of a marker

- 9 -

selected from the group including the markers listed in
Tables 1-5 (murine) or 7-10 (human).

[0017]   In another embodiment, the invention provides a
5   kit for diagnosing a subject with multiple sclerosis,
the kit including an antibody which specifically binds
with a protein corresponding to a marker selected from
the group including the markers listed in Tables 1-5
(murine) or 7-10 (human).
10

[0018]   In another embodiment, the invention provides a
method of modulating the level of expression of a
marker selected from the markers listed in Tables 1-5
(murine) or 7-10 (human), the method comprising
15   providing to diseased cells of the subject an antisense
oligonucleotide complementary to a polynucleotide
corresponding to the marker.

[0019]   In yet another embodiment, the invention
20   provides a method of modulating the level of expression
of a marker selected from the markers listed in Tables
1-5 (murine) or 7-10 (human), the method comprising
providing to diseased cells of a subject a protein.   In
a particularly preferred embodiment, the invention
25   further provides a vector which comprises a
polynucleotide encoding the protein.

[0020]   In another embodiment, the invention provides a
method of modulating a level of expression of a marker
30   selected from the markers listed in Tables 1-5 (murine)
or 7-10 (human), where the method comprises providing
to diseased cells of a subject an antibody.   In a
particularly preferred embodiment, the method further
comprises a therapeutic moiety conjugated to the
35   antibody.

[0021] In another preferred embodiment, the invention
provides a method of localizing a therapeutic moiety to
diseased tissue of a subject comprising exposing the
tissue to an antibody which is specific to a protein
5   encoded by a marker listed in Tables 1-5 (murine) or 7-
10 (human).

[0022] In another preferred embodiment, the present
invention provides a method of screening for a test
10   compound capable of modulating the activity of a
protein encoded from a marker listed in Tables 1-5
(murine) or 7-10 (human), said method comprising
combining said protein and test compound, and
determining the effect of said test compound on the
15   therapeutic efficacy of said protein.

[0023] In yet another preferred embodiment, the
present invention provides a method of screening for a
bioactive agent capable of interfering with the binding
20   of a protein or a fragment thereof and an antibody
which binds to said protein or fragment thereof, where
the method combines a protein or fragment thereof, a
bioactive agent and an antibody which binds to the
protein or fragment thereof, wherein the method further
25   includes determining the binding of the protein or
fragment thereof and the antibody.

[0024] In another preferred embodiment, the present
invention provides an antibody which specifically binds
30   to a protein encoded from a marker listed in Tables 1-5
(murine) or 7-10 (human). In particularly preferred
embodiment, the antibody is monoclonal and humanized.

- 11 -

[0026]   In yet another preferred embodiment, the
present invention provides a peptide encoded from
markers listed in Tables 1-5 (murine) or 7-10 (human).
Furthermore, the present invention is also directed to
5   a composition comprising the peptide.

[0027]   In an alternative embodiment, the present
invention provides a composition capable of modulating
an immune response in a subject, where the composition
10   comprises a protein encoded from a marker listed in
Tables 1-5 (murine) or 7-10 (human) and a
pharmaceutically acceptable carrier.

[0028]   In yet another embodiment, the present
15   invention provides a biochip comprising a panel of
markers selected from the group of markers listed in
Tables 1-5 (murine) or 7-10 (human). Furthermore, in a
particularly preferred embodiment, the markers for a
biochip may be selected for subjects suspected of
20   having multiple sclerosis from different stages of the
disease: secondary progressive, primary progressive,
relapsing-remitting. In a still another embodiment,
the markers may be selected for a subject which is from
a higher-risk geographical region.
25

[0029] Other features and advantages of the invention
will be apparent from the following detailed
description and claims.

30   BRIEF DESCRIPTION OF THE DRAWINGS

[0030]   FIGURE 1A through 1D depict the relative
expression levels of four cytokines, IL-10(FIGURE 1A;
$p < 0.4$), IL-8(FIGURE 1B; $p < 0.027$), IL-12p35(FIGURE 1C;

- 12 -

p<0.028) and IL-1β(FIGURE 1D; p<0.011), in MS-afflicted
subjects versus nondiseased subjects, as measured in
Example 2(D) below. The sample size was 10 MS-afflicted
subjects and 10 nondiseased subjects.

5

[0031] FIGURE 2 depicts the results of the class
predictor used in Example 2(E), wherein a diagnosis of
"normal" is indicated as positive and "MS-afflicted"
are indicated as negative. The model was based on
10 markers listed in Table 10, as expressed in human blood
samples.

         DETAILED DESCRIPTION OF THE INVENTION
[0032] The present invention provides methods for
15 diagnosis and prognosis evaluation for multiple
sclerosis (MS) in subjects, as well as methods and
molecular targets for therapeutic intervention.

[0033] In one aspect of the invention, the expression
20 levels of genes are determined in a particular patient
sample for which either diagnosis or prognosis
information is desired. The level of expression of a
number of genes simultaneously provides an expression
profile, which is essentially a "fingerprint" of the
25 activity of a gene or plurality of genes that is unique
to the state of the cell. Comparison of relative
levels of expression have been found to be indicative
of the presence of multiple sclerosis, and as such
permits for diagnostic and prognostic analysis. By
30 comparing relative expression profiles of multiple
sclerosis tissue in known different states (i.e.
secondary progressive vs. primary progressive vs.
relapsing-remitting), information regarding which genes
are important (including both up- and down-regulation
35 of genes) in each of these states is obtained. The

- 13 -

identification of gene markers that are differentially
expressed in diseased versus non-diseased tissue, as
well as differential expression resulting in different
prognostic outcomes, allows the use of this invention
5   in a number of ways. For example, the evaluation of a
particular treatment regime may be evaluated: will a
particular drug act to improve the long-term prognosis
in a particular patient? The discovery of these
differential expression patterns for individual genes
10   allows for screening of drug candidates with an eye to
mimicking or altering a particular expression pattern;
for example, screening can be done for drugs that will
alter the MS differential expression pattern or convert
a poor prognosis pattern to a better prognosis pattern.
15   This may be done by making biochips comprising sets of
the significant MS genes, which can then be used in
these screens. These methods can also be done on the
protein basis; that is protein expression levels of the
MS-associated proteins can be evaluated for diagnostic
20   and prognostic purposes or to screen test compounds.
In addition, the markers can be administered for gene
therapy purposes, including the administration of
antisense nucleic acids, or proteins (including
antibodies and other modulators thereof) administered
25   as therapeutic drugs.

[0034] Moreover, in a preferred embodiment, the
relative expression levels are measured from human
samples; however, as will be appreciated by those in
30   the art, expression levels of markers from other
organisms may be useful in animal models of disease and
drug evaluation; thus other markers are provided, from
vertebrates, including mammals, including rodents
(mice, hamsters, rats, guinea pigs, etc), primates,
35   farm animals (including sheep, goats, pigs, cows,

- 14 -

horses, etc). Markers from other organisms may be
obtained using the techniques outlined below.

[0035] The present invention is based, at least in
5   part, on the identification of a number of genetic
markers, set forth in Tables 1-5 (mouse) and 7-10
(human), which are differentially expressed between
diseased samples (MS-associated) and non-diseased
samples. Using a murine model of experimental
10 autoimmune encephalitis ("EAE") as an analogy to human
MS, a panel of 11,000 known murine genes was screened
for expression in diseased versus non-diseased tissue
from twelve different mice afflicted with the disease
(see Example 1). Those genes with statistically
15 substantial differences between the diseased and normal
tissues are identified in Tables 1-2. This
differential expression was observed either as an
increase in expression (Table 1), or a decrease in
expression (Table 2). In addition, to narrow the
20 subset of diseased-related, immune-mediated genes,
diseased cells were stimulated *in vitro* with a growth
protein and with an inhibitory compound, to yield 6
genes which were differentially expressed: CAPN12
(calpain 12), MT1 (metallothioneln 1), MYO1F (myosin
25 11), TLN (talin), UNK_AA117532 (EST), and UNK_AA645990
(EST).

[0036] Using the murine model as a springboard, a
panel of 12,000 human genes was likewise screened for
30 differential expression between diseased samples of the
brain and blood versus non-diseased samples (either
from noninvolved brain tissue or from non-diseased
subjects) (see Example 2). Genes that were
differentially up- or down- regulated in MS blood
35 (peripheral blood mononuclear cells, or "PBMCs")

- 15 -

samples are shown in Table 7, while genes that were
differentially up- or down-regulated in MS brain
samples are shown in Table 8. Furthermore, comparison
of the differentially expressed genes between blood and
5   brain samples yielded 181 genes in common. Further
comparison of the 181 genes to the murine genes that
were differentially regulated *in vitro* yielded six
genes (shown in Table 9 with their accession numbers):
EEF1D, PIM2, PRDX2, SEC24C, UNK_AJ24 AND XIP.

10

[0037] Included among the genes used to screen diseased
versus non-diseased tissue in the murine panel were two
genes known in the art to be implicated in EAE: TNF-α
and IFN-β. These genes served as an internal control.
15   Each of these genes were found to be substantially
increased in expression in EAE cells as opposed to non-
diseased cells, thus validating the method as a means
for identifying significant genes involved in EAE
pathology. Correspondingly, the genes which are known
20   in the art to be linked to MS (listed in Table 6) may
also serve as validation in expression studies for MS.
Moreover, the differentially regulated genes of the
invention, as listed in Tables 1-5 (murine) or 7-10
(human), have not been previously associated with EAE
25   or multiple sclerosis.

[0038] Accordingly, the present invention pertains to
the use of the genes set forth in Tables 1-5 (murine)
or 7-10 (human), the corresponding mRNA transcripts,
30   and the encoded polypeptides as markers for the
presence or risk of development of MS. These markers
are further useful to correlate the extent and/or
severity of disease. In particular, the present
invention is directed to the genes set forth in Table 9
35   (genes which were shown to be differentially regulated

- 16 -

in both murine and human models of MS), and in Table
10, the genes which were used in the MS class predictor
of Example 2(E). Panels of the markers can be
conveniently arrayed on solid supports, i.e. biochips
5   for use in kits. Markers can also be useful for
assessing the efficacy of a treatment or therapy of MS.

[0039] In one aspect, the invention provides markers
whose level of expression, which signifies their
10   quantity or activity, is correlated with the presence
of MS. The markers of the invention may be nucleic
acid molecules (e.g., DNA, cDNA or mRNA) or peptide(s).
Preferably the invention is performed by detecting the
presence of a transcribed polynucleotide or a portion
15   thereof, wherein the transcribed polynucleotide
comprises the marker. Alternatively, detection may be
performed be detecting the presence of a protein which
corresponds to the marker. The markers of the
invention are either increased or decreased in quantity
20   or activity in MS tissue as compared to non-diseased
tissue. For example, the gene designated 'SAA3' is
increased in expression level in murine EAE cells,·
relative to control cells, while the gene designated
'E_TC36651_s" is decreased in expression level in
25   murine EAE cells, relative to control cells. Both the
presence of increased or decreased mRNA for these genes
(and for other genes set forth in Tables 1-5 and 7-10),
and also increased or decreased levels of the protein
products of these genes (and other genes set forth in
30   Tables 1-5 and 7-10) serve as markers for either EAE or
MS. Preferably, increased or decreased levels of the
markers of the invention are increases and decreases of
a magnitude that are statistically substantial as
compared to appropriate control samples (i.e., non-

- 17 -

involved tissue or from non-diseased subjects.) In
particularly preferred embodiments, the marker is
increased or decreased relative to control samples by
at least 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, or 10- fold or
5   more. Similarly one skilled in the art will be
cognizant of the fact that a preferred detection
methodology is one in which the resulting detection
values are above the minimum detection limit of the
methodology.
10

[0040] Detection and measurement of the relative
amount of a nucleic acid or peptide marker of the
invention may be by any method known in the art (see,
i.e., Sambrook, J., Fritsh, E.F., and Maniatis, T.
15   *Molecular Cloning: A Laboratory Manual.* 2nd, ed., *Cold
Spring Harbor Laboratory*, Cold Spring Harbor Laboratory
Press, Cold Spring Harbor, NY (1989), and *Current
Protocols in Molecular Biology*, eds. Ausubel et al,
John Wiley & Sons (1992)). Typical methodologies for
20   detection of a transcribed polynucleotide include RNA
extraction from a cell or tissue sample, followed by
hybridization of a labeled probe (i.e., a complementary
nucleic acid molecule) specific for the target RNA to
the extracted RNA and detection of the probe (i.e.
25   Northern blotting). Typical methodologies for peptide
detection include protein extraction from a cell or
tissue sample, followed by hybridization of a labeled
probe (i.e., an antibody) specific for the target
protein to the protein sample, and detection of the
30   probe. The label group can be a radioisotope, a
fluorescent compound, an enzyme, or an enzyme co-
factor. Detection of specific peptide(s) and nucleic
acid molecules may also be assessed by gel
electrophoresis, column chromatography, direct
35   sequencing, or quantitative PCR (in the case of nucleic

- 18 -

acid molecules) among many other techniques well known
to those skilled in the art.

[0041] In certain embodiments, the genes themselves
5   (i.e., the DNA or cDNA) may serve as markers for MS.
For example, the absence of nucleic acids corresponding
to a gene (i.e. a gene from Table 2) such as by
deletion of all or part of the gene, may be correlated
with disease. Similarly an increase of nucleic acid
10 corresponding to a gene (i.e. a gene from Tables 1-5
and 7-10), such as by duplication of the gene, may also
be correlated with disease.

[0042] Detection of the presence or number of copies
15 of all or a part of a marker gene of the invention may
be performed using any method known in the art.
Typically, it is convenient to assess the presence
and/or quantity of a DNA or cDNA by Southern analysis,
in which total DNA from a cell or tissue sample is
20 extracted, is hybridized with a labeled probe (i.e. a
complementary DNA molecules), and the probe is
detected. The label group can be a radioisotope, a
fluorescent compound, an enzyme, or an enzyme co-
factor. Other useful methods of DNA detection and/or
25 quantification include direct sequencing, gel
electrophoresis, column chromatography, and
quantitative PCR, as is known by one skilled in the
art.

30 [0043] The invention also encompasses nucleic acid and
peptide molecules which are structurally different from
the molecules described above (i.e. which have a slight
altered nucleic acid or amino acid sequence), but which
have the same properties as the molecules above (e.g.,
35 encoded amino acid sequences, or which are changed only

- 19 -

in nonessential amino acid residues). Such molecules include allelic variants, and are described in greater detail in subsection I.

5   [0044]   In another aspect, the invention provides markers whose quantity or activity is correlated with different stages of MS: secondary progressive, primary progressive, relapsing-remitting. These markers are either increased or decreased in quantity or activity 10   in MS tissue in a fashion that is either positively or negatively correlated with the degree of severity of the MS. A method of monitoring progression of MS in subjects may be devised by detecting a substantial difference between the levels of expression in a 15   diseased subject at different points in time. The subsequent level of expression may further be compared to different expression profiles of various MS stages to confirm whether the subject has a matching profile. In yet another aspect, the invention provides markers 20   whose quantity or activity is correlated with a risk in a subject for developing MS. For instance, the markers may be selected for higher-risk geographical regions. These markers are either increased or decreased in activity or quantity in direct correlation to the 25   likelihood of the development of MS in a subject.

[0045]   Each marker may be considered individually, although it is within the scope of the invention to provide combinations of two or more markers for use in 30   the methods and compositions of the invention to increase the confidence of the analysis. In another aspect, the invention provides panels of the markers of the invention. In a preferred embodiment, these panels of markers are selected such that the markers within 35   any one panel share certain features (see Example

2(E)). For example, the markers of a first panel may
each exhibit a decrease in quantity or activity in MS`
tissue as compared to samples from non-involved samples
from the same subject or tissue from a non-diseased

5   subject. Similarly, different panels of markers may be
composed of markers from different tissues (i.e., blood
(Table 7) or brain tissue (Table 8)), or may represent
different components of an MS stage or type (i.e.,
secondary progressive, primary progressive, relapsing-

10   remitting in MS humans; or onset, peak and recovery in
EAE mice). Panels of the markers of the invention may
be made by independently selecting markers from any of
Tables 1-5 for mice, and Tables 7-10 for humans, and
may further be provided on biochips, as discussed

15   below.

[0046] It will be appreciated by one skilled in the art
that the panels of markers of the invention may
conveniently be provided on solid supports, as a

20   biochip. For example, polynucleotides may be coupled
to an array (e.g., a biochip using GeneChip® for
hybridization analysis), to a resin (e.g., a resin
which can be packed into a column for column
chromatography), or a matrix (e.g. a nitrocellulose

25   matrix for northern blot analysis). The immobilization
of molecules complementary to the marker(s), either
covalently or noncovalently, permits a discrete
analysis of the presence or activity of each marker in
a sample. In an array, for example, polynucleotides

30   complementary to each member of a panel of markers may
individually be attached to different, known locations
on the array. The array may be hybridized with, for
example, polynucleotides extracted from a brain sample
from a subject. The hybridization of polynucleotides

35   from the sample with the array at any location on the

- 21 -

array can be detected, and thus the presence or
quantity of the marker in the sample can be
ascertained. In a preferred embodiment, an array based
on a biochip is employed. Similarly, Western analyses
5   may be performed on immobilized antibodies specific for
different polypeptide markers hybridized to a protein
sample from a subject.

[0047] It will also be apparent to one skilled in the
10   art that the entire marker protein or nucleic acid
molecule need not be conjugated to the biochip support;
a portion of the marker or sufficient length for
detection purposes (i.e., for hybridization), for
example a portion of the marker which is 7, 10, 15, 20,
15   25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 100 or more
nucleotides or amino acids in length may be sufficient
for detection purposes.

[0048] The nucleic acid and peptide markers of the
20   invention may be isolated from any tissue or cell of a
subject. In a preferred embodiment, the tissue is
brain tissue. However, it will be apparent to one
skilled in the art that other tissue samples, including
bodily fluids such as blood, may also serve as sources
25   from which the markers of the invention may be
assessed. The tissue samples containing one or more of
the markers themselves may be useful in the methods of
the invention, and one skilled in the art will be
cognizant of the methods by which such samples may be
30   conveniently obtained, stored and/or preserved.

[0049] Several markers were known prior to the
invention to be associated with MS and are provided in
Table 6. These markers are not to be considered as

- 22 -

markers of the invention. However, these markers may
be conveniently be used in combination with the markers
of the invention (Tables 1-5 and 7-10) in the methods,
panels and kits of the invention.

5

[0050] In another aspect, the invention provides
methods of making an isolated hybridoma which produces
an antibody useful for diagnosing a patient with MS.
In this method, a protein corresponding to a marker of
10   the invention is isolated (e.g., by purification from a
cell in which it is expressed or by transcription and
translation of a nucleic acid encoding the protein *in
vivo* or *in vitro* using known methods). A vertebrate,
preferably a mammal such as a mouse, rabbit or sheep,
15   is immunized using the isolated protein or protein
fragment. The vertebrate may optionally (and
preferably) be immunized at least one additional time
with the isolated protein or protein fragment, so that
the vertebrate exhibits a robust immune response to the
20   protein or protein fragment. Splenocytes are isolated
from the immunized vertebrate and fused with an
immortalized cell line to form hybridomas, using any of
a variety of methods well known in the art. Hybridomas
formed in this manner are then screened using standard
25   methods to identify one or more hybridomas which
produce an antibody which specifically binds with the
protein or protein fragment. The invention also
includes hybridomas made by this method and antibodies
made using such hybridomas.

30

[0051] The invention provides methods of diagnosing MS,
or determining the risk of developing MS. These
methods involve isolating a sample from a subject
(e.g., a sample containing blood cells or brain cells),
35   detecting the presence, quantity and/or activity of one

- 23 -

or more markers of the invention in the sample relative
to a second sample from a non-diseased subject, or from
a non-involved tissue in the same subject. The levels
of markers in the two samples are compared, and a

5   substantial increase or decrease in one or more markers
in the test sample indicates the presence or risk of
presence of MS in the subject.

[0052]   The invention also provides methods of

10   assessing the efficacy of a test compound or therapy
for inhibiting MS in a subject. These methods involve
isolating samples from a subject suffering from MS who
is undergoing treatment or therapy, and detecting the
presence, quantity, and/or activity of one or more

15   markers of the invention in the first sample relative
to a second sample. Where a test compound is
administered, the first and second samples are
preferably sub-portions of a single sample taken from
the patient, wherein the first portion is exposed to

20   the test compound and the second portion is not. In
one aspect of this embodiment, the substantially
different level of expression is a substantially lower
level of expression in the first sample, relative to
the second. Most preferably, the level of expression

25   in the first sample approximates (i.e., less than a two
fold difference from a control) the level of expression
in a third control sample, taken from either a non-
diseased subject or non-involved tissue.

30   [0053]   Where the efficacy of a therapy is being
assessed, the first sample obtained from the subject is
preferably obtained prior to provision of at least a
portion of the therapy, whereas the second sample is
obtained following provision of the portion of the

35   therapy. The levels of markers in the samples are

- 24 -

compared, preferably against a third control sample as
well, and correlated with the presence, risk of
presence, or severity of MS. Most preferably, the
level of markers in the second sample approximates the
5   level of expression of a third control sample. By
assessing whether expression of MS has been lessened or
alleviated in the sample, the ability of the treatment
or therapy to treat MS is determined.

10 [0054]   The invention also provides a method of
screening test compounds for inhibitors of MS, and to
the pharmaceutical compositions comprising the test
compounds. The method of screening comprises obtaining
samples of diseased or involved cells, maintaining
15 separate aliquots of the samples with a plurality of
test compounds, and comparing expression of a marker in
each of the aliquots to determine whether any of the
test compounds provides a substantially different level
of expression from a control. In addition, methods of
20 screening may be devised by combining a test compound
with a protein and thereby determining the effect of
the test compound on the protein. Alternatively, the
invention is further directed to a method of screening
for bioactive agents capable of interfering with the
25 binding of a protein encoded by the markers of Tables
1-5 (murine) or 7-10 (human), and an antibody, by
combining the bioactive agent, protein, and antibody
together and determining whether binding of the
antibody and protein occurs.
30

[0055]   Moreover, the invention is directed to
pharmaceutical compositions comprising the test
compound, or bioactive agent, which may further include
a marker protein and/or nucleic acid of the invention
35 (e.g., for those markers in Tables 1-5 or 7-10 which

- 25 -

are decreased or increased in quantity or activity in
MS versus non-diseased tissue), and can be formulated
as described herein. Alternatively, these compositions
may include an antibody which specifically binds to a
5   marker protein of the invention and/or an antisense
nucleic acid molecule which is complementary to a
marker nucleic acid of the invention (e.g., for those
markers which are increased in quantity in MS tissue)
and can be formulated as described herein.
10

[0056]   The invention further provides methods of
modulating a level of expression of a marker of the
invention, comprising administration to the diseased
cells of the subject a variety of compositions which
15   correspond to the markers of Tables 1-5 (murine) or 7-
10 (human), including proteins or antisense
oligonucleotides. The protein may be provided to the
diseased cells by further providing a vector comprising
a polynucleotide encoding the protein to the cells.
20   Alternatively, the expression levels of the markers of
the invention may be modulated by providing an
antibody, a plurality of antibodies or an antibody
conjugated to a therapeutic moiety. Treatment with the
antibody may further be localized to the diseased
25   tissue. In another aspect, the invention provides
methods for localizing a therapeutic moiety to diseased
tissue comprising exposing the tissue to an antibody
which is specific to a protein encoded from the markers
of the invention. This method may therefore provide a
30   means to inhibit or enhance expression of a specific
gene corresponding to a marker listed in Tables 1-5 or
7-10. Where the gene is up-regulated as a result of MS
pathology, it is likely that inhibition of MS
progression would involve inhibiting expression of the
35   up-regulated gene. As a corollary to this method,

- 26 -

where the gene is down-regulated, inhibition of MS
progression would therefore likely require enhancing
expression of the down-regulated gene.

5   [0057]   In another aspect, the invention includes
antibodies that are specific to proteins corresponding
to markers of the invention.   Preferably the antibodies
are monoclonal, and most preferably, the antibodies are
humanized, as per the description of antibodies
10   described below.

[0058]   In still another aspect of the invention, the
invention includes peptides or proteins which are
encoded from the markers of the invention, and to
15   compositions thereof.

[0059]   The invention also provides kits for diagnosing
a subject with MS, the kit comprising reagents for
assessing expression of the markers of the invention.
20   Preferably, the reagents may be an antibody or fragment
thereof, wherein the antibody or fragment thereof
specifically binds with a protein corresponding to a
marker from Tables 1-5 or 7-10.   Optionally, the kits
may comprise a nucleic acid probe wherein the probe
25   specifically binds with a transcribed polynucleotide
corresponding to a marker selected from the group
consisting of the markers listed in Tables 1-5 or 7-10.

30   [0060]   The invention further provides kits for
assessing the suitability of each of a plurality of
compounds for inhibiting progression of MS in a
subject.   Such kits include a plurality of compounds to
be tested, and a reagent (i.e. antibody specific to

- 27 -

corresponding proteins of the invention) for assessing
expression of a marker listed in Tables 1-5 or 7-10.

[0061]   Modifications to the above-described
5   compositions and methods of the invention, according to
standard techniques, will be readily apparent to one
skilled in the art and are meant to be encompassed by
the invention.

10   [0062]   To facilitate an understanding of the present
invention, a number of terms and phrases are defined
below:

[0063]   As used herein, the term "modulation" includes,
15   in its various grammatical forms (e.g., "modulated",
"modulation", "modulating", etc.), up-regulation,
induction, stimulation, potentiation, and/or relief of
inhibition, as well as inhibition and/or down-
regulation.
20

[0064]   As used herein, the terms "polynucleotide" and
"oligonucleotide" are used interchangeably, and include
polymeric forms of nucleotides of any length, either
deoxyribonucleotides or ribonucleotides, or analogs
25   thereof. Polynucleotides may have any three-
dimensional structure, and may perform any function,
known or unknown. The following are non-limiting
examples of polynucleotides: a gene or gene fragment,
exons, introns, messenger RNA (mRNA), transfer RNA,
30   ribosomal RNA, ribozymes, cDNA, recombinant
polynucleotides, branched polynucleotides, plasmids,
vectors, isolated DNA of any sequence, isolated RNA of
any sequence, nucleic acid probes, and primers. A
polynucleotide may comprise modified nucleotides, such

- 28 -

as methylated nucleotides and nucleotide analogs. If
present, modifications to the nucleotide structure may
be imparted before or after assembly of the polymer.
The sequence of nucleotides may be interrupted by non-
5   nucleotide components. A polynucleotide may be further
modified after polymerization, such as by conjugation
with a labeling component. The term also includes both
double- and single-stranded molecules. Unless
otherwise specified or required, any embodiment of this
10 invention that is a polynucleotide encompasses both the
double-stranded form and each of two complementary
single-stranded forms known or predicted to make up the
double-stranded form.

15 [0065]   A polynucleotide is composed of a specific
sequence of four nucleotide bases: adenine (A);
cytosine(C); guanine (G); thymine (T); and uracil (U)
for guanine when the polynucleotide is RNA. This, the
term "polynucleotide sequence" is the alphabetical
20 representation of a polynucleotide molecule. This
alphabetical representation can be inputted into
databases in a computer having a central processing
unit and used for bioinformatics applications such as
functional genomics and homology searching.

25

[0066]   A "gene" includes a polynucleotide containing
at least one open reading frame that is capable of
encoding a particular polypeptide or protein after
being transcribed and translated. Any of the
30 polynucleotide sequences described herein may be used
to identify larger fragments or full-length coding
sequences of the gene with which they are associated.
Methods of isolating larger fragment sequences are
known to those of sill in the art, some of which are
35 described herein.

- 29 -

[0067] A "gene product" includes an amino acid sequence(e.g., peptide or polypeptide) generated when a gene is transcribed and translated.

[0068] As used herein, a "polynucleotide corresponds to" another (a first) polynucleotide if it is related to the first polynucleotide by any of the following relationships:

1) The second polynucleotide comprises the first polynucleotide and the second polynucleotide encodes a gene product.

2) The second polynucleotide is 5' or 3' to the first polynucleotide in cDNA, RNA, genomic DNA, or fragments of any of these polynucleotides. For example, a second polynucleotide may be a fragment of a gene that includes the first and second polynucleotides. The first and second polynucleotides are related in that they are components of the gene coding for a gene product, such as a protein or antibody. However, it is not necessary that the second polynucleotide comprises or overlaps with the first polynucleotide to be encompassed within the definition of "corresponding to" as used herein. For example, the first polynucleotide may be a fragment of a 3' untranslated region of the second polynucleotide. The first and second polynucleotide may be fragments of a gene coding for a gene product. The second polynucleotide may be an exon of the gene while the first polynucleotide may be an intron of the gene.

- 30 -

3) The second polynucleotide is the complement of the
first polynucleotide.

[0069] As used herein, the term, "transcribed" or
5   "transcription" refers to the process by which genetic
code information is transferred from one kind of
nucleic acid to another, and refers in particular to
the process by which a base sequence of mRNA is
synthesized on a template of cDNA.
10

[0070] A "probe" when used in the context of
polynucleotide manipulation includes an oligonucleotide
that is provided as a reagent to detect a target
present in a sample of interest by hybridizing with the
15   target. Usually, a probe will comprise a label or a
means by which a label can be attached, either before
or subsequent to the hybridization reaction. Suitable
labels include, but are not limited to radioisotopes,
fluorochromes, chemiluminescent compounds, dyes, and
20   proteins, including enzymes.

[0071] A "primer" includes a short polynucleotide,
generally with a free 3'-OH group that binds to a
target or "template" present in a sample of interest by
25   hybridizing with the target, and thereafter promoting
polymerization of a polynucleotide complementary to the
target. A "polymerase chain reaction" ("PCR") is a
reaction in which replicate copies are made of a target
polynucleotide using a "pair of primers" or "set or
30   primers" consisting of "upstream" and a "downstream"
primer, and a catalyst of polymerization, such as a DNA
polymerase, and typically a thermally-stable polymerase
enzyme. Methods for PCR are well known in the art, and
are taught, for example, in MacPherson et al., IRL
35   Press at Oxford University Press (1991)). All

- 31 -

processes of producing replicate copies of a
polynucleotide, such as PCR or gene cloning, are
collectively referred to herein as "replication".
A primer can also be used as a probe in hybridization
5   reactions, such as Southern or Northern blot analyses
(see, e.g., Sambrook, J., Fritsh, E.F., and
Maniatis, T. *Molecular Cloning: A Laboratory Manual.*
2nd, ed., Cold Spring Harbor Laboratory, Cold Spring
Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).
10

[0072]   The term "cDNAs" includes complementary DNA,
that is mRNA molecules present in a cell or organism
made into cDNA with an enzyme such as reverse
transcriptase.   A "cDNA library" includes a collection
15   of mRNA molecules present in a cell or organism,
converted into cDNA molecules with the enzyme reverse
transcriptase, then inserted into "vectors" (other DNA
molecules that can continue to replicate after addition
of foreign DNA).   Exemplary vectors for libraries
20   include bacteriophage, viruses that infect bacteria
(e.g., lambda phage).   The library can then be probed
for the specific cDNA (and thus mRNA) of interest.

[0073]   A "gene delivery vehicle" includes a molecule
25   that is capable of inserting one or more
polynucleotides into a host cell.   Examples of gene
delivery vehicles are liposomes, biocompatible
polymers, including natural polymers and synthetic
polymers; lipoproteins; polypeptides; polysaccharides;
30   lipopolysaccharides; artificial viral envelopes; metal
particles; and bacteria, viruses and viral vectors,
such as baculovirus, adenovirus, and retrovirus,
bacteriophage, cosmid, plasmid, fungal vector and other
recombination vehicles typically used in the art which

- 32 -

have been described for replication and/or expression
in a variety of eukaryotic and prokaryotic hosts. The
gene delivery vehicles may be used for replication of
the inserted polynucleotide, gene therapy as well as
5   for simply polypeptide and protein expression.

[0074]   A "vector" includes a self-replicating nucleic
acid molecule that transfers an inserted polynucleotide
into and/or between host cells. The term is intended
10 to include vectors that function primarily for
insertion of a nucleic acid molecule into a cell,
replication vectors that function primarily for the
replication of nucleic acid and expression vectors that
function for transcription and/or translation of the
15 DNA or RNA. Also intended are vectors that provide
more than one of the above function.

[0075]   A "host cell" is intended to include any
individual cell or cell culture which can be or has
20 been a recipient for vectors or for the incorporation
of exogenous nucleic acid molecules, polynucleotides
and/or proteins. It also is intended to include
progeny of a single cell. The progeny may not
necessarily be completely identical (in morphology or
25 in genomic or total DNA complement) to the original
parent cell due to natural, accidental, or deliberate
mutation. The cells may be prokaryotic or eukaryotic,
and include but are not limited to bacterial cells,
yeast cells, insect cells, animal cells, and mammalian
30 cells, e.g., murine, rat, simian or human cells.

[0076]   The term "genetically modified" includes a cell
containing and/or expressing a foreign gene or nucleic
acid sequence which in turn modifies the genotype or

- 33 -

phenotype of the cell or its progeny. This term
includes any addition, deletion, or disruption to a
cell's endogenous nucleotides.

5   [0077] As used herein, "expression" includes the
process by which polynucleotides are transcribed into
mRNA and translated into peptides, polypeptides, or
proteins. If the polynucleotide is derived from
genomic DNA, expression may include splicing of the
10   mRNA, if an appropriate eukaryotic host is selected.
Regulatory elements required for expression include
promoter sequences to bind RNA polymerase and
transcription initiation sequences for ribosome
binding. For example, a bacterial expression vector
15   includes a promoter such as the lac promoter and for
transcription initiation the Shine-Dalgarno sequence
and the start codon AUG (Sambrook, J., Fritsh, E. F.,
and Maniatis, T. *Molecular Cloning: A Laboratory
Manual. 2nd, ed., Cold Spring Harbor Laboratory*, Cold
20   Spring Harbor Laboratory Press, Cold Spring Harbor, NY,
1989). Similarly, a eukaryotic expression vector
includes a heterologous or homologous promoter for RNA
polymerase II, a downstream polyadenylation signal, the
start codon AUG, and a termination codon for detachment
25   of the ribosome. Such vectors can be obtained
commercially or assembled by the sequences described in
methods well known in the art, for example, the methods
described below for constructing vectors in general.

30   [0078] "Differentially expressed", as applied to a
gene, includes the differential production of mRNA
transcribed from a gene or a protein product encoded by
the gene. A differentially expressed gene may be
overexpressed or underexpressed as compared to the
35   expression level of a normal or control cell. In one

- 34 -

aspect, it includes a differential that is 2 times, preferably 5 times or preferably 10 times higher or lower than the expression level detected in a control sample. The term "differentially expressed" also
5   includes nucleotide sequences in a cell or tissue which are expressed where silent in a control cell or not expressed where expressed in a control cell.

[0079]   The term "polypeptide" includes a compound of
10 two or more subunit amino acids, amino acid analogs, or peptidomimetics. The subunits may be linked by peptide bonds. In another embodiment, the subunit may be linked by other bonds, e.g., ester, ether, etc. As used herein the term "amino acid" includes either
15 natural and/or unnatural or synthetic amino acids, including glycine and both the D or L optical isomers, and amino acid analogs and peptidomimetics. A peptide of three or more amino acids is commonly referred to as an oligopeptide. Peptide chains of greater than three
20 or more amino acids are referred to as a polypeptide or a protein.

[0080]   "Hybridization" includes a reaction in which one or more polynucleotides react to form a complex
25 that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson-Crick base pairing, Hoogstein binding, or in any other sequence-specific manner. The complex may comprise two strands forming a duplex
30 structure, there or more strands forming a multi-stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of a PCR reaction, or the enzymatic
35 cleavage of a polynucleotide by a ribozyme.

[0081]   Hybridization reactions can be performed under
conditions of different "stringency". The stringency
of a hybridization reaction includes the difficulty
5   with which any two nucleic acid molecules will
hybridize to one another. The present invention also
includes polynucleotides capable of hybridizing under
reduced stringency conditions, more preferably
stringent conditions, and most preferably highly
10   stringent conditions, to polynucleotides described
herein. Examples of stringency conditions are shown in
Table A below: highly stringent conditions are those
that are at least as stringent as, for example,
conditions A-F; stringent conditions are at least as
15   stringent as, for example, conditions G-L; and reduced
stringency conditions are at least as stringent as, for
example, conditions M-R.

- 36 -

Table A. Stringency Conditions

Stringency Condition	Poly-nucleotide Hybrid	Hybrid Length (bp)[1]	Hybridization Temperature and Buffer[H]	Wash Temperature and Buffer[H]
A	DNA:DNA	> 50	65°C; 1xSSC -or- 42°C; 1xSSC, 50% formamide	65°C; 0.3xSSC
B	DNA:DNA	<50	T_B^*; 1xSSC	T_B^*; 1xSSC
C	DNA:RNA	> 50	67°C; 1xSSC -or- 45°C; 1xSSC, 50% formamide	67°C; 0.3xSSC
D	DNA:RNA	<50	T_D^*; 1xSSC	T_D^*; 1xSSC
E	RNA:RNA	>50	70°C; 1xSSC -or- 50°C; 1xSSC, 50% formamide	70°C; 0.3xSSC
F	RNA:RNA	<50	T_F^*; 1xSSC	T_F^*; 1xSSC
G	DNA:DNA	> 50	65°C; 4xSSC -or- 42°C; 4xSSC, 50% formamide	65°C; 1xSSC
H	DNA:DNA	<50	T_H^*; 4xSSC	T_H^*; 4xSSC
I	DNA:RNA	> 50	67°C; 4xSSC -or- 45°C; 4xSSC, 50% formamide	67°C; 1xSSC
J	DNA:RNA	<50	T_J^*; 4xSSC	T_J^*; 4xSSC
K	RNA:RNA	> 50	70°C; 4xSSC -or- 50°C; 4xSSC, 50% formamide	67°C; 1xSSC
L	RNA:RNA	<50	T_L^*; 2xSSC	T_L^*; 2xSSC
M	DNA:DNA	> 50	50°C; 4xSSC -or- 40°C; 6xSSC, 50% formamide	50°C; 2xSSC
N	DNA:DNA	<50	T_N^*; 6xSSC	T_N^*; 6xSSC
O	DNA:RNA	> 50	55°C; 4xSSC -or- 42°C; 6xSSC, 50% formamide	55°C; 2xSSC
P	DNA:RNA	<50	T_P^*; 6xSSC	T_P^*; 6xSSC
Q	RNA:RNA	> 50	60°C; 4xSSC -or- 45°C; 6xSSC, 50% formamide	60°C; 2xSSC
R	RNA:RNA	<50	T_R^*; 4xSSC	T_R^*; 4xSSC

1: The hybrid length is that anticipated for the hybridized region(s) of the hybridizing polynucleotides. When hybridizing a polynucleotide to a target polynucleotide of unknown sequence, the hybrid length is assumed to be that of the hybridizing polynucleotide. When polynucleotides of known sequence are hybridized, the hybrid length can be determined by aligning the sequences of the polynucleotides and identifying the region or regions of optimal sequence complementarity.

H: SSPE (1xSSPE is 0.15M NaCl, 10mM NaH_2PO_4, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes after hybridization is complete.

T_B^* - T_R^*: The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, $T_m(°C) = 2(\text{# of A + T bases}) + 4(\text{# of G + C bases})$. For hybrids between 18 and 49 base pairs in length, $T_m(°C) = 81.5 + 16.6(\log_{10}Na^+) + 0.41(\%G+C) - (600/N)$, where N is the number of bases in the hybrid, and Na^+ is the concentration of sodium ions in the hybridization buffer (Na^+ for 1xSSC = 0.165 M).

- 37 -

Additional examples of stringency conditions for
polynucleotide hybridization are provided in Sambrook,
J., E.F. Fritsch, and T. Maniatis, 1989, Molecular
Cloning: A Laboratory Manual, Cold Spring Harbor
5   Laboratory Press, Cold Spring Harbor, NY, chapters 9
and 11, and Current Protocols in Molecular Biology,
1995, F.M. Ausubel et al., eds., John Wiley & Sons,
Inc., sections 2.10 and 6.3-6.4, incorporated herein by
reference.
10

[0082]   When hybridization occurs in an antiparallel
configuration between two single-stranded
polynucleotides, the reaction is called "annealing" and
those polynucleotides are described as "complementary".
15 A double-stranded polynucleotide can be "complementary"
or "homologous" to another polynucleotide, if
hybridization can occur between one of the strands of
the first polynucleotide and the second.
"Complementarity" or "homology" (the degree that one
20 polynucleotide is complementary with another) is
quantifiable in terms of the proportion of bases in
opposing strands that are expected to hydrogen bond
with each other, according to generally accepted base-
pairing rules.
25

[0083]   An "antibody" includes an immunoglobulin
molecule capable of binding an epitope present on an
antigen. As used herein, the term encompasses not only
intact immunoglobulin molecules such as monoclonal and
30 polyclonal antibodies, but also anti-idotypic
antibodies, mutants, fragments, fusion proteins,
bi-specific antibodies, humanized proteins, and
modifications of the immunoglobulin molecule that
comprises an antigen recognition site of the required
35 specificity.

- 38 -

[0084]   As used herein, the term "diseased" refers to
cells, tissues or samples from a subject afflicted with
multiple sclerosis, wherein the cell, tissue or sample

5   has been affected by multiple sclerosis (i.e. from
white-matter lesion). As used herein, the term "non-
diseased" refers to cells, tissues or other such
samples taken from a subject who is not afflicted with
multiple sclerosis. As used herein, "non-involved"

10   refers to cells, tissues, or samples wherein the tissue
is from a subjected afflicted with MS, but wherein the
cells, tissues or samples are believed to be unaffected
by multiple sclerosis. Preferred tissue (and cell)
samples are from brain, blood, sera, lymph, thymus,

15   spleen, bone marrow or pus. Most preferred samples are
peripheral blood mononuclear cells ("PBMC") or brain
tissue.

[0085]   As used herein, the term "marker" includes a
polynucleotide or polypeptide molecule which is present

20   or absent, or increased or decreased in quantity or
activity in subjects afflicted with multiple sclerosis,
or in MS-associated cells. The relative change in
quantity or activity of the marker is correlated with
the incidence or risk of incidence of multiple

25   sclerosis.

[0086]   As used herein, the term "panel of markers"
includes a group of markers, the quantity or activity
of each member of which is correlated with the

30   incidence or risk of incidence of a MS-associated
condition. In certain embodiments, a panel of markers
may include only those markers which are either
increased or decreased in quantity or activity in
subjects afflicted with or cells involved in a MS-

35   associated condition.   In a preferred embodiment, the

- 39 -

panel of markers comprises at least 5 markers, and most
preferably, the panel comprises markers listed in Table
10. In other embodiments, a panel of markers may
include only those markers present in a specific tissue
5 type which are correlated with the incidence of risk of
incidence of a MS-associated condition.

Various aspects of the invention are described in
further detail in the following subsections:
10

I.   Isolated Nucleic Acid Molecules

[0087]   One aspect of the invention pertains to
isolated nucleic acid molecules that either themselves
15 are the genetic markers (e.g., mRNA) of the invention,
or which encode the polypeptide markers of the
invention, or fragments thereof. Another aspect of the
invention pertains to isolated nucleic acid fragments
sufficient for sue as hybridization probes to identify
20 the nucleic acid molecules encoding the markers for the
invention in a sample, as well as nucleotide fragments
for use as PCR primers of the amplification or mutation
of the nucleic acid molecules which encode the markers
of the invention. As used herein, the term "nucleic
25 acid molecule" is intended to include DNA molecules
(e.g., cDNA or genomic DNA) and RNA molecules (e.g.,
mRNA) and analogs of the DNA or RNA generated using
nucleotide analogs. The nucleic acid molecule can be
single-stranded or double-stranded, but preferably is
·30 double-stranded DNA..

[0088]   The term "isolated nucleic acid molecule"
includes nucleic acid molecules which are separated
from other nucleic acid molecules which are present in
35 the natural source of the nucleic acid. For example,

- 40 -

with regards to genomic DNA, the term "isolated" includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated"

5   nucleic acid is free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated marker

10   nucleic acid molecule of the invention, or nucleic acid molecule encoding a polypeptide marker of the invention, can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid

15   molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques,

20   or substantially free of chemical precursors or other chemicals when chemically synthesized.

[0089] A nucleic acid molecule of the present invention, e.g., a nucleic acid molecule having the

25   nucleotide sequence of one of the genes set forth in Tables 1-5 or 7-10, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or portion of the nucleic acid sequence of one of

30   the genes set forth in Tables 1-5 or 7-10 as a hybridization probe, a marker gene of the invention or a nucleic acid molecule encoding a polypeptide marker of the invention can be isolated using standard hybridization and cloning techniques (e.g., as

35   described in Sambrook, J., Fritsh, E. F., and Maniatis,

- 41 -

T. *Molecular Cloning: A Laboratory Manual 2nd, ed.,
Cold Spring Harbor Laboratory,* Cold Spring Harbor
Laboratory Press, Cold spring Harbor, NY, 1989).

5   [0090]   A nucleic acid of the invention can be
amplified using cDNA, mRNA or alternatively, genomic
DNA, as a template and appropriate oligonucleotide
primers according to standard PCR amplification
techniques. The nucleic acid so amplified can be
10   cloned into an appropriate vector and characterized by
DNA sequence analysis. Furthermore, oligonucleotides
corresponding to marker nucleotide sequences, or
nucleotide sequences encoding a marker of the invention
can be prepared by standard synthetic techniques, *e.g.,*
15   using an automated DNA synthesizer.

[0091]   In another preferred embodiment, an isolated
nucleic acid molecule of the invention comprises a
nucleic acid molecule which is a complement of the
20   nucleotide sequence of a marker of the invention (e.*g.,*
a gene set forth in Tables 1-5 or 7-10), or a portion
of any of these nucleotide sequences. A nucleic acid
molecule which is complementary to such a nucleotide
sequence is one which is sufficiently complementary t
25   the nucleotide sequence such that it can hybridize to
the nucleotide sequence, thereby forming a stable
duplex.

[0092]   The nucleic acid molecule of the invention,
30   moreover, can comprise only a portion of the nucleic
acid sequence of a marker nucleic acid of the
invention, or a gene encoding a marker polypeptide of
the invention, for example, a fragment which can be
used as a probe or primer. The probe/primer typically

- 42 -

comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 7 or 15, preferably about
5   20 or 25, more preferably about 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 400 or more consecutive nucleotides of a marker nucleic acid, or a nucleic acid encoding a marker polypeptide of the invention.
10

[0093]   Probes based on the nucleotide sequence of a marker gene or of a nucleic acid molecule encoding a marker polypeptide of the invention can be used to detect transcripts or genomic sequences corresponding
15   to the marker gene(s) and/or marker polypeptide(s) of the invention.   In preferred embodiments, the probe comprises a label group attached thereto, e.g., the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor.   Such
20   probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress (e.g., over- or under-express) a marker polypeptide of the invention, or which have greater or fewer copies of a marker gene of the invention.   For example, a level of
25   a marker polypeptide-encoding nucleic acid in a sample of cells from a subject may be detected, the amount of mRNA transcript of a gene encoding a marker polypeptide may be determined, or the presence of mutations or deletions of a marker gene of the invention may be
30   assessed.

[0094]   The invention further encompasses nucleic acid molecules that differ from the nucleic acid sequences of the genes set forth in Tables 1-5 or 7-10, due to
35   degeneracy of the genetic code and which thus encode

- 43 -

the same proteins as those encoded by the genes shown
in Tables 1-5 or 7-10.

[0095]   In addition to the nucleotide sequences of the
5   genes set forth in Tables 1-5 or 7-10, it will be
appreciated by those skilled in the art that DNA
sequence polymorphisms that lead to changes in the
amino acid sequences of the proteins encoded by the
genes set forth in Tables 1-5 or 7-10 may exist within
10   a population e.g., the human population). Such genetic
polymorphism in the genes set forth in Tables 1-5 or 7-
10 may exist among individuals within a population due
to natural allelic variation. An allele is one of a
group of genes which occur alternatively at a given
15   genetic locus. In addition it will be appreciated that
DNA polymorphisms that affect RNA expression levels can
also exist that may affect the overall expression level
of that gene e.g., by affecting regulation or
degradation). As used herein, the phrase "allelic
20   variant" includes a nucleotide sequence which occurs ta
a given locus or to a polypeptide encoded by the
nucleotide sequence. As used herein, the terms "gene"
and "recombinant gene" refer to nucleic acid molecules
which include an open reading frame encoding a marker
25   polypeptide of the invention.

[0096]   Nucleic acid molecules corresponding to natural
allelic variants and homologues of the marker genes, or
genes encoding the marker proteins of the invention can
30   be isolated based on their homology to the genes set
forth in Tables 7-10, using the cDNAs disclosed herein,
or a portion thereof, as a hybridization probe
according to standard hybridization techniques under
stringent hybridization conditions. Nucleic acid
35   molecules corresponding to natural allelic variants and

- 44 -

homologues of the marker genes of the invention can
further be isolated by mapping to the same chromosome
or locus as the marker genes or genes encoding the
marker proteins of the invention.

5

[0097]   In another embodiment, an isolated nucleic acid
molecule of the invention is at least 15, 20, 25, 30,
50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550,
600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100,
10   1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000 or
more nucleotides in length and hybridizes under
stringent conditions to a nucleic acid molecule
corresponding to a nucleotide sequence of a marker gene
or gene encoding a marker protein of the invention. As
15   used herein, the term "hybridizes under stringent
conditions" is intended to describe conditions for
hybridization and washing under which nucleotide
sequences at least 60% homologous to each other
typically remain hybridized to each other. Preferably,
20   the conditions are such that sequences at least about
70%, more preferably at least about 80%, even more
preferably at least about 85% or 90% homologous to each
other typically remain hybridized to each other. Such
stringent conditions are known to those skilled in the
25   art and can be found in *Current Protocols in Molecular
Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6.
Preferably, an isolated nucleic acid molecule of the
invention that hybridizes under stringent conditions to
the sequence of one of the genes set forth in Tables 1-
30   5 or 7-10 corresponds to a naturally-occurring nucleic
acid molecule. As used herein, a "naturally-occurring"
nucleic acid molecule includes an RNA or DNA molecule
having a nucleotide sequence that occurs in nature
(*e.g.*, encodes a natural protein).

35

- 45 -

[0098]   In addition to naturally-occurring allelic
variants of the marker gene and gene encoding a marker
protein of the invention sequences that may exist in
the population, the skilled artisan will further
5   appreciate that changes can be introduced by mutation
into the nucleotide sequences of the marker genes or
genes encoding the marker proteins of the invention,
thereby leading to changes in the amino acid sequence
of the encoded proteins, without altering the
10   functional activity of these proteins.   For example,
nucleotide substitutions leading to amino acid
substitutions at "non-essential" amino acid residues
can be made.   A "non-essential" amino acid residue is a
residue that can be altered from the wild-type sequence
15   of a protein without altering the biological activity,
whereas an "essential" amino acid residue is required
for biological activity.   For example, amino acid
residues that are conserved among allelic variants or
homologs of a gene (e.g., among homologs of a gene from
20   different species) are predicted to be particularly
unamenable to alteration.

[0099]   Accordingly, another aspect of the invention
pertains to nucleic acid molecules encoding a marker
25   protein of the invention that contain changes in amino
acid residues that are not essential for activity.
Such proteins differ in amino acid sequence from the
marker proteins encoded by the genes set forth in
Tables 1-5 or 7-10, yet retain biological activity.   In
30   one embodiment, the protein comprises an amino acid
sequence at least about 60%, 65%, 70%, 75%, 80%, 85%,
90%, 95%, 98% or more homologous to a marker protein of
the invention.

- 46 -

[0100]   An isolated nucleic acid molecule encoding a
protein homologous to a marker protein of the invention
can be created by introducing one or more nucleotide
substitutions, additions or deletions into the
5   nucleotide sequence of the gene encoding the marker
protein, such that one or more amino acid
substitutions, additions or deletions are introduced
into the encoded protein. Mutations can be introduced
into the genes of the invention (e.g., a gene set forth
10   in Tables 7-10) by standard techniques, such as site-
directed mutagenesis and PCR-mediated mutagenesis.
Preferably, conservative amino acid substitutions are
made at one or more predicted non-essential amino acid
residues. A "conservative amino acid substitution" is
15   one in which the amino acid residue is replaced with an
amino acid residue having a similar side chain.
Families of amino acid residues having similar side
chains have been defined in the art. These families
include amino acids with basic side chains (e.g.,
20   lysine, arginine, histidine), acidic side chains (e.g.,
aspartic acid, glutamic acid), uncharged polar side
chains (e.g., glycine, asparagine, glutamine, serine,
threonine, tyrosine, cysteine), nonpolar side chains
(e.g., alanine, valine, leucine, isoleucine, proline,
25   phenylalanine, methionine, tryptophan), beta-branched
side chains (e.g., threonine, valine, isoleucine) and
aromatic side chains (e.g., tyrosine, phenylalanine,
tryptophan, histidine). Alternatively, mutations can
be.introduced randomly along all or part of a coding
30   sequence of a gene of the invention, such as by
saturation mutagenesis, and the resultant mutants can
be screened for biological activity to identify mutants
that retain activity. Following mutagenesis, the

encoded protein can be expressed recombinantly and the
activity of the protein can be determined.

[0101]   Another aspect of the invention pertains to
5   isolated nucleic acid molecules which are antisense to
the marker genes and genes encoding marker proteins of
the invention. An "antisense" nucleic acid comprises a
nucleotide sequence which is complementary to a "sense"
nucleic acid encoding a protein, e.g., complementary to
10   the coding strand of a double-stranded cDNA molecule or
complementary to an mRNA sequence. Accordingly, an
antisense nucleic acid can hydrogen bond to a sense
nucleic acid. The antisense nucleic acid can be
complementary to an entire coding strand of a gene of
15   the invention (e.g., a gene set forth in Tables 1-5 or
7-10), or to only a portion thereof. In one
embodiment, an antisense nucleic acid molecule is
antisense to a "coding region" of the coding strand of
a nucleotide sequence of the invention. The term
20   "coding region" includes the region of the nucleotide
sequence comprising codons which are translated into
amino acid. In another embodiment, the antisense
nucleic acid molecule is antisense to a "noncoding
region" of the coding strand of a nucleotide sequence
25   of the invention.

[0102]   The term "noncoding region" includes 5' and 3'
sequences which flank the coding region that are not
translated into amino acids (i.e., also referred to as
30   5' and 3' untranslated regions).

[0103]   Antisense nucleic acids of the invention can be
designed according to the rules of Watson and Crick
base pairing. The antisense nucleic acid molecule can

- 48 -

be complementary to the entire coding region of an mRNA
corresponding to a gene of the invention, but more
preferably is an oligonucleotide which is antisense to
only a portion of the coding or noncoding region. An

5   antisense oligonucleotide can be, for example, about 5,
10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in
length. An antisense nucleic acid of the invention can
be constructed using chemical synthesis and enzymatic
ligation reactions using procedures known in the art.

10   For example, an antisense nucleic acid (e.g., an
antisense oligonucleotide) can be chemically
synthesized using naturally occurring nucleotides or
variously modified nucleotides designed to increase the
biological stability of the molecules or to increase

15   the physical stability of the duplex formed between the
antisense and sense nucleic acids, e.g.,
phosphorothioatc derivatives and acridine substituted
nucleotides can be used. Examples of modified
nucleotides which can be used to generate the antisense

20   nucleic acid include 5-fluorouracil, 5-bromouracil, 5-
chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-
acetylcytosine, 5-(carboxyhydroxyhnethyl) uracil, 5-
carboxymethylaminomethyl-2-thiouridine,
5-carboxymethylaminomethyluracil, dihydrouracil, beta-

25   D-galactosylqueosine, inosine, N6-isopentenyladenine,
1-methylguanine, 1-methylinosine, 2,2-dimethylguanine,
2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-
methylcytosine, N6-adenine, 7-methylguanine, 5-
methylaminomethyluracil, 5-methoxyaminomethyl-2-

30   thiouracil, beta-D-mannosylqueosine, 5'-
methoxycarboxymethyluracil, 5-methoxyuracil, 2-
methylthio-N6-isopentenyladen4exine, unacil-5-oxyacetic
acid (v), wybutoxosine, pseudouracil, queosine, 2-
thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-

35   thiouracil, 5-methyluracil, uracil-5- oxyacetic acid

- 49 -

methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-
thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil,
(acp3)w, and 2,6-diaminopurine. Alternatively, the
antisense nucleic acid can be produced biologically
5   using an expression vector into which a nucleic acid
has been subcloned in an antisense orientation (i.e.,
RNA transcribed from the inserted nucleic acid will be
of an antisense orientation to a target nucleic acid of
interest, described further in the following
10 subsection).

[0104] The antisense nucleic acid molecules of the
invention are typically administered to a subject or
generated in situ such that they hybridize with or bind
15 to cellular mRNA and/or genomic DNA encoding a marker
protein of the invention to thereby inhibit expression
of the protein, e.g., by inhibiting transcription
and/or translation. The hybridization can be by
conventional nucleotide complementarity to form a
20 stable duplex, or, for example, in the cases of an
antisense nucleic acid molecule which binds to DNA
duplexes, through specific interactions in the major
groove of the double helix. An example of a route of
administration of antisense nucleic acid molecules of
25 the invention include direct injection at a tissue site
(e.g., in brain). Alternatively, antisense nucleic
acid molecules can be modified to target selected cells
and then administered systemically. For example, for
systemic administration, antisense molecules can be
30 modified such that they specifically bind to receptors
or antigens expressed on a selected cell surface, e.g.,
by linking the antisense nucleic acid molecules to
peptides or antibodies which bind to cell surface
receptors or antigens. The antisense nucleic acid
35 molecules can also be delivered to cells using the

- 50 -

vectors described herein. To achieve sufficient
intracellular concentrations of the antisense
molecules, vector constructs in which the antisense
nucleic acid molecule is placed under the control of a
5 strong pol II or pol III promoter are preferred.

[0105] In yet another embodiment, the antisense
nucleic acid molecule of the invention is an α-anomeric
nucleic acid molecule. An α-anomeric nucleic acid
10 molecule forms specific double-stranded hybrids with
complementary RNA in which, contrary to the usual β-
units, the strands run parallel to each other (Gaultier
et al. (1987) *Nucleic Acids. Res.* 15:6625-6641). The
antisense nucleic acid molecule can also comprise a 2'-
15 o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic
Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue
(Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

[0106] In still another embodiment, an antisense
20 nucleic acid of the invention is a ribozyme. Ribozymes
are catalytic RNA molecules with ribonuclease activity
which are capable of cleaving a single-stranded nucleic
acid, such as an mRNA, to which they have a
complementary region. Thus, ribozymes (*e.g.*,
25 hammerhead ribozymes (described in Haselhoif and
Gerlach (1988) *Nature* 334:585-591)) can be used to
catalytically cleave mRNA transcripts of the genes of
the invention (*e.g.*, a gene set forth in Tables 1-5 or
7-10) to thereby inhibit translation of this mRNA. A
30 ribozyme having specificity for a marker protein-
encoding nucleic acid can be designed based upon the
nucleotide sequence of a gene of the invention,
disclosed herein. For example, a derivative of a
Tetrahymena L-19 IVS RNA can be constructed in which

- 51 -

the nucleotide sequence of the active site is
complementary to the nucleotide sequence to be cleaved
in a marker protein-encoding mRNA. See, *e.g.*, Cech *et
al.* U.S. Patent No. 4,987,071; and Cech *et al.* U.S.
5   Patent No. 5,116,742. Alternatively, mRNA transcribed
from a gene of the invention can be used to select a
catalytic RNA having a specific ribonuclease activity
from a pool of RNA molecules. See, *e.g.*, Bartel, D.
and Szostak, J.W. (1993) *Science* 261:1411-1418.
10

[0107] Alternatively, expression of a gene of the
invention (*e.g.*, a gene set forth in Tables 1-5 or 7-
10) can be inhibited by targeting nucleotide sequences
complementary to the regulatory region of these genes
15   (*e.g.*, the promoter and/or enhancers) to form triple
helical structures that prevent transcription of the
gene in target cells. See generally, Helene, C. (1991)
Anticancer Drug Des. 6(6):569-84; Helene, C. *et al.*
(1992). *Ann. N. Y. Acad Sci.* 660:27-36; and Maher, L.J.
20   (1992) *Bioassays* 14(12):807-15.

[0108] Expression of the marker genes, and genes
encoding marker proteins of the invention, can also be
inhibited using RNA interference ("RNA_i"). This is a
25   technique for post transcriptional gene silencing
("PTGS"), in which target gene activity is specifically
abolished with cognate double-stranded RNA ("dsRNA").
RNA_i resembles in many aspects PTGS in plants and has
been detected in many invertebrates including
30   trypanosome, hydra, planaria, nematode and fruit fly
(Drosophila melanogaster). It may be involved in the
modulation of transposable element mobilization and
antiviral state formation. RNA_i in mammalian systems is
disclosed in PCT application WO 00/63364 which is

- 52 -

incorporated by reference herein in its entirety.
Basically, dsRNA of at least about 600 nucleotides,
homologous to the target marker is introduced into the
cell and a sequence specific reduction in gene activity
5   is observed. See generally, Ui-Teia, K. et al. *FEBS
Letters* 479: 79-82.

[0109]   In yet another embodiment, the nucleic acid
molecules of the present invention can be modified at
10 the base moiety, sugar moiety or phosphate backbone to
improve, *e.g.*, the stability, hybridization, or
solubility of the molecule. For example, the
deoxyribose phosphate backbone of the nucleic acid
molecules can be modified to generate peptide nucleic
15 acids (see Hyrup B. *et al.* (1996) *Bioorganic &
Medicinal Chemistry* 4(1): 5 23). As used herein, the
terms "peptide nucleic acids" or "PNAs" refer to
nucleic acid mimics, *e.g.*, DNA mimics, in which the
deoxyribose phosphate backbone is replaced by a
20 pseudopeptide backbone and only the four natural
nucleobases are retained. The neutral backbone of PNAs
has been shown to allow for specific hybridization to
DNA and RNA under conditions of low ionic strength.
The synthesis of PNA oligomers can be performed using
25 standard solid phase peptide synthesis protocols as
described in Hyrup B. *et al.* (1996) *supra*; Perry-
O'Keefe *et al.* Proc. Natl. Acad. Sci. 93: 14670-675.

[0110]   PNAs can be used in therapeutic and diagnostic
30 applications. For example, PNAs can be used as
antisense or antigene agents for sequence-specific
modulation of gene expression by, for example, inducing
transcription or translation arrest or inhibiting
replication. PNAs of the nucleic acid molecules of the

- 53 -

invention (*e.g.*, a gene set forth in Tables 1-5 or 7-
10) can also be used in the analysis of single base
pair mutations in a gene, (*e.g.*, by PNA-directed PCR
clamping); as 'artificial restriction enzymes' when
5   used in combination with other enzymes, (*e.g.*, S1
nucleases (Hyrup B. (1996) *supra*)); or as probes or
primers for DNA sequencing or hybridization (Hyrup B.
et al. (1996) *supra*; Perry-O'Keefe *supra*).

10   [0111]   In another embodiment, PNAs can be modified,
(*e.g.*, to enhance their stability or cellular uptake),
by attaching lipophilic or other helper groups to PNA,
by the formation of PNA-DNA chimeras, or by the use of
liposomes or other techniques of drug delivery known in
15   the art.   For example, PNA-DNA chimeras of the nucleic
acid molecules of the invention can be generated which
may combine the advantageous properties of PNA and DNA.
Such chimeras allow DNA recognition enzymes, (*e.g.*,
RNAse H and DNA polymerases), to interact with the DNA
20   portion while the PNA portion would provide high
binding affinity and specificity.   PNA-DNA chimeras can
be linked using linkers of appropriate lengths selected
in terms of base stacking, number of bonds between the
nucleobases, and orientation (Hyrup B. (1996) *supra*).
25   The synthesis of PNA-DNA chimeras can be performed as
described in Hyrup B. (1996) *supra* and Finn P.J. *et al.*
(1996) *Nucleic Acids Res.* 24 (17): 3357-63.   For
example, a DNA chain can be synthesized on a solid
support using standard phosphoramidite coupling   .
30   chemistry and modified nucleoside analogs, *e.g.*, 5'-(4-
methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite,
can be used as a between the PNA and the 5' end of DNA
(Mag, M. *et al.* (1989) *Nucleic Acid Res.* 17: 5973-88).
PNA monomers are then coupled in a stepwise manner to

- 54 -

produce a chimeric molecule with a 5' PNA segment and a
3' DNA segment (Finn P.J. et al. (1996) *supra*).
Alternatively, chimeric molecules can be synthesized
with a 5' DNA segment and a 3' PNA segment (Peterser,
5   K.H. et al. (1975) *Bioorganic Med Chem. Lett.* 5: 1119-
11124).

[0112]   In other embodiments, the oligonucleotide may
include other appended groups such as peptides (*e.g.*,
10   for targeting host cell receptors *in vivo*), or agents
facilitating transport across the cell membrane (see,
e.g., Letsinger et al. (1989) *Proc. Natl. Acad. Sci.
USA* 86:6553-6556; Lemaitre et al. (1987) *Pros. Natl.
Acad Sci. USA* 84:648-652; PCT Publication No.
15   WO88/09810) or the blood-brain barrier (see, *e.g.*, PCT
Publication No. WO89/10134).   In addition,
oligonucleotides can be modified with hybridization-
triggered cleavage agents (See, *e.g.*, Krol et al.
(1988) *Bio-Techniques* 6:958-976) or intercalating
20   agents. (See, *e.g.*, Zon (1988) *Pharm. Res.* 5:539-549).
To this end, the oligonucleotide may be conjugated to
another molecule, (*e.g.*, a peptide, hybridization
triggered cross-linking agent, transport agent, or
hybridization-triggered cleavage agent).   Finally, the
25   oligonucleotide may be detectably labeled, either such
that the label is detected by the addition of another
reagent (*e.g.*, a substrate for an enzymatic label), or
is detectable immediately upon hybridization of the
nucleotide (*e.g.*, a radioactive label or a fluorescent
30   label (*e.g.*, a molecular beacon, as described in U.S.
Patent 5,876,930).

- 55 -

II. Isolated Proteins and Antibodies

[0113] One aspect of the invention pertains to
isolated marker proteins, and biologically active
5   portions thereof, as well as polypeptide fragments
suitable for use as immunogens to raise anti-marker
protein antibodies. In one embodiment, native marker
proteins can be isolated from cells or tissue sources
by an appropriate purification scheme using standard
10   protein purification techniques. In another
embodiment, marker proteins are produced by recombinant
DNA techniques. Alternative to recombinant expression,
a marker protein or polypeptide can be synthesized
chemically using standard peptide synthesis techniques.
15

[0114] An "isolated" or "purified" protein or
biologically active portion thereof is substantially
free of cellular material or other contaminating
proteins from the cell or tissue source from which the
20   marker protein is derived, or substantially free from
chemical precursors or other chemicals when chemically
synthesized. The language "substantially free of
cellular material" includes preparations of marker
protein in which the protein is separated from cellular
25   components of the cells from which it is isolated or
recombinantly produced. In one embodiment, the
language "substantially free of cellular material"
includes preparations of marker protein having less
than about 30% (by dry weight) of non-marker protein
30   (also referred to herein as a "contaminating protein"),
more preferably less than about 20% of non-marker
protein, still more preferably less than about 10% of
non-marker protein, and most preferably less than about
5% non-marker protein. When the marker protein or
35   biologically active portion thereof is recombinantly

- 56 -

produced, it is also preferably substantially free of
culture medium, i.e., culture medium represents less
than about 20%, more preferably less than about 10%,
and most preferably less than about 5% of the volume of
5   the protein preparation.

[0115]   The language "substantially free of chemical
precursors or other chemicals" includes preparations of
marker protein in which the protein is separated from
10 chemical precursors or other chemicals which are
involved in the synthesis of the protein.   In one
embodiment, the language "substantially free of
chemical precursors or other chemicals" includes
preparations of protein having less than about 30% (by
15 dry weight) of chemical precursors or non-protein
chemicals, more preferably less than about 20% chemical
precursors or non-protein chemicals, still more
preferably less than about 10% chemical precursors or
non-protein chemicals, and most preferably less than
20 about 5% chemical precursors or non-protein chemicals.

[0116]   As used herein, a "biologically active portion"
of a marker protein includes a fragment of a marker
protein comprising amino acid sequences sufficiently
25 homologous to or derived from the amino acid sequence
of the marker protein, which include fewer amino acids
than the full length marker proteins, and exhibit at
least one activity of a marker protein.   Typically,
biologically active portions comprise a domain or motif
30 with at least one activity of the marker protein.   A
biologically active portion of a marker protein can be
a polypeptide which is, for example, 10, 25, 50, 100,
200 or more amino acids in length.   Biologically active
portions of a marker protein can be used as targets for

- 57 -

developing agents which modulate a marker protein-
mediated activity.

[0117]   In a preferred embodiment, marker protein is
5   encoded by a gene set forth in Tables 1-5 or 7-10. In
other embodiments, the marker protein is substantially
homologous to a marker protein encoded by a gene set
forth in Tables 1-5 or 7-10, and retains the functional
activity of the marker protein, yet differs in amino
10 acid sequence due to natural allelic variation or
mutagenesis, as described in detail in subsection I
above. Accordingly, in another embodiment, the marker
protein is a protein which comprises an amino acid
sequence at least about 60%, 65%, 70%, 75%, 80%, 85%,
15 90%, 95%, 98% or more homologous to the amino acid   '
sequence encoded by a gene set forth in Tables 1-5 or
7-10.

[0118]   To determine the percent identity of two amino
20 acid sequences or of two nucleic acid sequences, the
sequences are aligned for optimal comparison purposes
(e.g., gaps can be introduced in one or both of a first
and a second amino acid or nucleic acid sequence for
optimal alignment and non-homologous sequences can be
25 disregarded for comparison purposes). In a preferred
embodiment, the length of a reference sequence aligned
for comparison purposes is at least 30%, preferably at
least 40%, more preferably at least 50%, even more
preferably at least 60%, and even more preferably at
30 least 70%, 80%, or 90% of the length of the reference
sequence. The amino acid residues or nucleotides at
corresponding amino acid positions or nucleotide
positions are then compared. When a position in the
first sequence is occupied by the same amino acid
35 residue or nucleotide as the corresponding position in

- 58 -

the second sequence, then the molecules are identical
at that position (as used herein amino acid or nucleic
acid "identity" is equivalent to amino acid or nucleic
acid "homology"). The percent identity between the two
5   sequences is a function of the number of identical
positions shared by the sequences, taking into account
the number of gaps, and the length of each gap, which
need to be introduced for optimal alignment of the two
sequences.
10

[0119] The comparison of sequences and determination
of percent identity between two sequences can be
accomplished using a mathematical algorithm. In a
preferred embodiment, the percent identity between two
15   amino acid sequences is determined using the Needleman
and Wunsch (*J. Mot. Biol.* (48):444-453 (1970))
algorithm which has been incorporated into the GAP
program in the GCG software package (available at
http://www.gcg.com), using either a Blossom 62 matrix
20   or a PAM250 matrix, and a gap weight of 16, 14, 12, 10,
8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.
In yet another preferred embodiment, the percent
identity between two nucleotide sequences is determined
using the GAP program in the GCG software package
25   (available at http://www.gcg.com), using a
NWSgapdna.CMP matrix and a gap weight of 40, 50, 60,
70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6.
In another embodiment, the percent identity between two
amino acid or nucleotide sequences is determined using
30   the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-
17 (1989)) which has been incorporated into the ALIGN
program (version 2.0), using a PAM 120 weight residue
table, a gap length penalty of 12 and a gap penalty of
4.

35

- 59 -

[0120]   The nucleic acid and protein sequences of the
present invention can further be used as a "query
sequence" to perform a search against public databases
to, for example, identify other family members or
5   related sequences. Such searches can be performed
using the NBLAST and XBLAST programs (version 2.0) of
Altschul, et al. (1990) J. Mol. Biol. 215:403-10.
BLAST nucleotide searches can be performed with the
NBLAST program, score = 100, wordlength = 12 to obtain
10   nucleotide sequences homologous to nucleic acid
molecules of the invention. BLAST protein searches can
be performed with the XBLAST program, score = 50,
wordlength = 3 to obtain amino acid sequences
homologous to marker protein molecules of the
15   invention. To obtain gapped alignments for comparison
purposes, Gapped BLAST can be utilized as described in
Altschul et al., (1997) Nucleic Acids Res. 25(17):3389-
3402. When utilizing BLAST and Gapped BLAST programs,
the default parameters of the respective programs
20   (e.g., XBLAST and NBLAST) can be used. See
http://www.ncbi.nim.nih.gov.

[0121]   The invention also provides chimeric or fusion
marker proteins. As used herein, a marker "chimeric
25   protein" or "fusion protein" comprises a marker
polypeptide operatively linked to a non-marker
polypeptide. An "marker polypeptide" includes a
polypeptide having an amino acid sequence encoded by a
gene set forth in Tables 1-5 or 7-10, whereas a "non-
30   marker polypeptide" includes a polypeptide having an
amino acid sequence corresponding to a protein which is
not substantially homologous to the marker protein,
e.g., a protein which is different from marker protein
and which is derived from the same or a different

- 60 -

organism. Within a marker fusion protein the
polypeptide can correspond to all or a portion of a
marker protein. In a preferred embodiment, a marker
fusion protein comprises at least one biologically
5   active portion of a marker protein. Within the fusion
protein, the term "operatively linked" is intended to
indicate that the marker polypeptide and the non-marker
polypeptide are fused in-frame to each other. The non-
marker polypeptide can be fused to the N-terminus or C-
10   terminus of the marker polypeptide.

[0122] For example, in one embodiment, the fusion
protein is a GST-marker fusion protein in which the
marker sequences are fused to the C-terminus of the GST
15   sequences. Such fusion proteins can facilitate the
purification of recombinant marker proteins.

[0123] In another embodiment, the fusion protein is a
marker protein containing a heterologous signal
20   sequence at its N-terminus. In certain host cells
(e.g., mammalian host cells), expression and/or
secretion of marker proteins can be increased through
use of a heterologous signal sequence. Such signal
sequences are well known in the art.
25

[0124] The marker fusion proteins of the invention can
be incorporated into pharmaceutical compositions and
administered to a subject in vivo, as described herein.
The marker fusion proteins can be used to affect the
30   bioavailability of a marker protein substrate. Use of
marker fusion proteins may be useful therapeutically
for the treatment of disorders (e.g., multiple
sclerosis) caused by, for example, (i) aberrant
modification or mutation of a gene encoding a marker

protein; (ii) mis-regulation of the marker protein-encoding gene; and (iii) aberrant post-translational modification of a marker protein.

5   [0125]   Moreover, the marker-fusion proteins of the invention can be used as immunogens to produce anti-marker protein antibodies in a subject, to purify marker protein ligands and in screening assays to identify molecules which inhibit the interaction of a
10   marker protein with a marker protein substrate.

[0126]   Preferably, a marker chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for
15   the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-
20   in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively,
25   PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example,
30   *Current Protocols In Molecular Biology,* eds. Ausubel *et al.* John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.,* a GST polypeptide). A marker protein-encoding nucleic acid
35   can be cloned into such an expression vector such that

the fusion moiety is linked in-frame to the marker
protein.

[0127] A signal sequence can be used to facilitate
secretion and isolation of the secreted protein or
other proteins of interest. Signal sequences are
typically characterized by a core of hydrophobic amino
acids which are generally cleaved from the mature
protein during secretion in one or more cleavage
events. Such signal peptides contain processing sites
that allow cleavage of the signal sequence from the
mature proteins as they pass through the secretory
pathway. Thus, the invention pertains to the described
polypeptides having a signal sequence, as well as to
polypeptides from which the signal sequence has been
proteolytically cleaved (*i.e.*, the cleavage products).
In one embodiment, a nucleic acid sequence encoding a
signal sequence can be operably linked in an expression
vector to a protein of interest, such as a protein
which is ordinarily not secreted or is otherwise
difficult to isolate. The signal sequence directs
secretion of the protein, such as from a eukaryotic
host into which the expression vector is transformed,
and the signal sequence is subsequently or concurrently
cleaved. The protein can then be readily purified from
the extracellular medium by art recognized methods.

[0128] Alternatively, the signal sequence can be
linked to the protein of interest using a sequence
which facilitates purification, such as with a GST
domain.

[0129] The present invention also pertains to variants
of the marker proteins of the invention which function

as either agonists (mimetics) or as antagonists to the
marker proteins. Variants of the marker proteins can
be generated by mutagenesis, e.g., discrete point
mutation or truncation of a marker protein. An agonist

5 of the marker proteins can retain substantially the
same, or a subset, of the biological activities of the
naturally occurring form of a marker protein. An
antagonist of a marker protein can inhibit one or more
of the activities of the naturally occurring form of

10 the marker protein by, for example, competitively
modulating an activity of a marker protein. Thus,
specific biological effects can be elicited by
treatment with a variant of limited function. In one
embodiment, treatment of a subject with a variant

15 having a subset of the biological activities of the
naturally occurring forth of the protein has fewer side
effects in a subject relative to treatment with the
naturally occurring form of the marker protein.

20 [0130] Variants of a marker protein which function as
either marker protein agonists (mimetics) or as marker
protein antagonists can be identified by screening
combinatorial libraries of mutants, e.g., truncation
mutants, of a marker protein for marker protein agonist

25 or antagonist activity. In one embodiment; a
variegated library of marker protein variants is
generated by combinatorial mutagenesis at the nucleic
acid level and is encoded by a variegated gene library.
A variegated library of marker protein variants can be

30 produced by, for example, enzymatically ligating a
mixture of synthetic oligonucleotides into gene
sequences such that a degenerate set of potential
marker protein sequences is expressible as individual
polypeptides, or alternatively, as a set of larger

35 fusion proteins (e.g., for phage display) containing

- 64 -

the set of marker protein sequences therein. There are
a variety of methods which can be used to produce
libraries of potential marker protein variants from a
degenerate oligonucleotide sequence. Chemical

5   synthesis of a degenerate gene sequence can be
performed in an automatic DNA synthesizer, and the
synthetic gene then ligated into an appropriate
expression vector. Use of a degenerate set of genes
allows for the provision, in one mixture, of all of the

10 sequences encoding the desired set of potential marker
protein sequences. Methods for synthesizing degenerate
oligonucleotides are known in the art (see, *e.g.*,
Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura *et al.*
(1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.*

15 (1984) *Science* 198:1055; Ike *et al.* (1983) *Nucleic Acid
Res.* 11:477).

[0131] In addition, libraries of fragments of a
protein coding sequence corresponding to a marker

20 protein of the invention can be used to generate a
variegated population of marker protein fragments for
screening and subsequent selection of variants of a
marker protein. In one embodiment, a library of coding
sequence fragments can be generated by treating a

25 double stranded PCR fragment of a marker protein coding
sequence with a nuclease under conditions wherein
nicking occurs only about once per molecule, denaturing
the double stranded DNA, renaturing the DNA to form
double stranded DNA which can include sense/antisense

30 pairs from different nicked products, ·removing single
stranded portions from reformed duplexes by treatment
with S1 nuclease, and ligating the resulting fragment
library into an expression vector. By this method, an
expression library can be derived which encodes N-

- 65 -

terminal, C-terminal and internal fragments of various
sizes of the marker protein.

[0132]   Several techniques are known in the art for
5   screening gene products of combinatorial libraries made
by point mutations or truncation, and for screening
cDNA libraries for gene products having a selected
property. The most widely used techniques, which are
amenable to high-throughput analysis, for screening
10   large gene libraries typically include cloning the gene
library into replicable expression vectors,
transforming appropriate cells with the resulting
library of vectors, and expressing the combinatorial
genes under conditions in which detection of a desired
15   activity facilitates isolation of the vector encoding
the gene whose product was detected. Recursive
ensemble mutagenesis (REM)., a new technique which
enhances the frequency of functional mutants in the
libraries, can be used in combination with the
20   screening assays to identify marker variants (Arkin and
Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815;
Delgrave *et al.* (1993) *Protein Engineering* 6(3):327-
331).

25   [0133]   An isolated marker protein, or a portion or
fragment thereof, can be used as an immunogen to
generate antibodies that bind marker proteins using
standard techniques for polyclonal and monoclonal
antibody preparation. A full-length marker protein can
30   be used or, alternatively, the invention provides
antigenic peptide fragments of these proteins for use
as immunogens. The antigenic peptide of a marker
protein comprises at least 8 amino acid residues of an
amino acid sequence encoded by a gene set forth in
35   Tables 1-5 or 7-10, and encompasses an epitope of a

- 66 -

marker protein such that an antibody raised against the
peptide forms a specific immune complex with the marker
protein. Preferably, the antigenic peptide comprises
at least 10 amino acid residues, more preferably at

5   least 15 amino acid residues, even more preferably at
least 20 amino acid residues, and most preferably at
least 30 amino acid residues.

[0134] Preferred epitopes encompassed by the antigenic
10   peptide are regions of the marker protein that are
located on the surface of the protein, *e.g.*,
hydrophilic regions, as well as regions with high
antigenicity.

15   [0135] A marker protein immunogen typically is used to
prepare antibodies by immunizing a suitable subject,
(*e.g.*, rabbit, goat, mouse or other mammal) with the
immunogen. An appropriate immunogenic preparation can
contain, for example, recombinantly expressed marker
20   protein or a chemically synthesized marker polypeptide.
The preparation can further include an adjuvant, such
as Freund's complete or incomplete adjuvant, or similar
immunostimulatory agent. Immunization of a suitable
subject with an immunogenic marker protein preparation
25   induces a polyclonal anti-marker protein antibody
response.

[0136] Accordingly, another aspect of the invention
pertains to anti-marker protein antibodies. The term
30   "antibody" as used herein includes immunoglobulin
molecules and immunologically active portions of
immunoglobulin molecules, i.e., molecules that contain
an antigen binding site which specifically binds
(immunoreacts with) an antigen, such as a marker

- 67 -

protein. Examples of immunologically active portions
of immunoglobulin molecules include F(ab) and F(ab')$_2$
fragments which can be generated by treating the
antibody with an enzyme such as pepsin. The invention
5   provides polyclonal and monoclonal antibodies that bind
to marker proteins. The term "monoclonal antibody" or
"monoclonal antibody composition", as used herein,
includes a population of antibody molecules that
contain only one species of an antigen binding site
10   capable of immunoreacting with a particular epitope. A
monoclonal antibody composition thus typically displays
a single binding affinity for a particular marker
protein with which it immunoreacts.

15   [0137] Polyclonal anti-marker protein antibodies can
be prepared as described above by immunizing a suitable
subject with a marker protein of the invention. The
anti-marker protein antibody titer in the immunized
subject can be monitored over time by standard
20   techniques, such as with an enzyme linked immunosorbent
assay (ELISA) using immobilized marker protein. If
desired, the antibody molecules directed against marker
proteins can be isolated from the mammal (e.g., from
the blood) and further purified by well known
25   techniques, such as protein A chromatography, to obtain
the IgG fraction. At an appropriate time after
immunization, e.g., when the anti-marker protein
antibody titers are highest, antibody-producing cells
can be obtained.from the subject and used to prepare
30   monoclonal antibodies by standard techniques, such as
the hybridoma technique originally described by Kohler
and Milstein (1975) Nature 256:495-497) (see also,
Brown et al. (1981) J. Immunol. 127:539-46; Brown et
al. (1980) J. Biol. Chem. 255:4980-83; Yeh et al.

- 68 -

(1976) *Proc. Natl. Acad, Sci. USA* 76:2927-3I; and Yeh
et al. (1982) *Int. J. Cancer* 29:269-75), the more
recent human B cell hybridoma technique (Kozbor *et al.*
(1983) *Immunol Today* 4:72), the EBV-hybridoma technique
5   (Cole *et al.* (1985), *Monoclonal Antibodies and Cancer
Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma
techniques. The technology for producing monoclonal
antibody hybridomas is well known (see generally R. H.
Kenneth, in *Monoclonal Antibodies: A New Dimension In
10   Biological Analyses*, Plenum Publishing Corp., New York,
New York (1980); E. A. Lerner (1981) *Yale J. Biol.
Med.*, 54:387-402; M.L. Gefter *et al.* (1977) *Somatic
Cell Genet.* 3:231-36). Briefly, an immortal cell line
(typically a myeloma) is fused to lymphocytes
15   (typically splenocytes) from a mammal immunized with a
marker protein immunogen as described above, and the
culture supernatants of the resulting hybridoma cells
are screened to identify a hybridoma producing a
monoclonal antibody that binds to a marker protein of
20   the invention.

[0138] Any of the many well known protocols used for
fusing lymphocytes and immortalized cell lines can be
applied for the purpose of generating an anti-marker
25   protein monoclonal antibody (see, *e.g.*, G. Galfre *et
al.* (1977) *Nature* 266:SSOS2; Gefter *et al. Somatic Cell
Genet.*, cited *supra*; Letter, *Yale J. Biol. Med.*, cited
supra; Kenneth, *Monoclonal Antibodies*, cited *supra*).
Moreover, the ordinarily skilled worker will appreciate
30   that there are many variations of such methods which
also would be useful. Typically, the immortal cell
line (*e.g.*, a myeloma cell line) is derived from the
same mammalian species as the lymphocytes. For
example, murine hybridomas can be made by fusing

- 69 -

lymphocytes from a mouse immunized with an immunogenic
preparation of the present invention with an
immortalized mouse cell line. Preferred immortal cell
lines are mouse myeloma cell lines that are sensitive
5 to culture medium containing hypoxanthine, axninopterin
and thymidine ("HAT medium"). Any of a number of
myeloma cell lines can be used as a fusion partner
according to standard techniques, *e.g.*, the P3-NS1/1-
Ag4-1, P3-x63-Ag8.653 or Sp210-Ag14 myeloma lines.
10 These myeloma lines are available from ATCC.
Typically, HAT-sensitive mouse myeloma cells are fused
to mouse splenocytes using polyethylene glycol ("PEG").
Hybridoma cells resulting from the fusion are then
selected using HAT medium, which kills unfused and
15 unproductively fused myeloma cells (unfused splenocytes
die after several days because they are not
transformed). Hybridoma cells producing a monoclonal
antibody of the invention are detected by screening the
hybridoma culture supernatants for antibodies that bind
20 to a marker protein, *e.g.*, using a standard ELISA
assay.

[0139]   Alternative to preparing monoclonal antibody-
secreting hybridomas, a monoclonal anti-marker protein
25 antibody can be identified and isolated by screening a
recombinant combinatorial immunoglobulin library (*e.g.*,
an antibody phase display library) with marker protein
to thereby isolate immunoglobulin library members that
bind to a marker protein. Kits for generating and
30 screening phage display libraries are commercially
available (*e.g.*, the Pharmacia *Recombinant Phage
Antibody System*, Catalog No. 27-9400-01; and the
Stratagene *SurfZAP*™ *Phage Display Kit*, Catalog No.
240612). Additionally, examples of methods and

- 70 -

reagents particularly amenable for use in generating
and screening antibody display library can be found in,
for example, Ladner *et al.* U.S. Patent No. 5,223,409;
Kang *et al.* PCT International Publication No. WO
5   92/18619; Dower *et al.* PCT International Publication
No. WO 91/17271; Winter *et al.* PCT International
Publication WO 92/20791; Markland *et al.* PCT
International Publication No. WO 92115679; Breitling *et
al.* PCT International Publication WO 93/01288;
10 McCafferty *et al.* PCT International Publication No. WO
92/01047; Garrard *et al.* PCT International Publication
No. WO 92/09690; Ladner *et al.* PCT International
Publication No. WO 90/02809; Fuchs *et al.* (1991)
Bio/Technology 9:1370-1372; Hay *et al.* (1992) *Hum.*
15 *Antibod Hybridomas* 3:81-85; Huse *et al.* (1989) *Science*
246:1275-1281; Griffiths *et al.* (1993) *EMBO J* 12:725-
734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896;
Clarkson *et al.* (1991) *Nature* 352:624-628; Gram *et
al.*.(1992) *Proc. Natl. Acad Sci. USA* 89:3576-3580;
20 Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377;
Hoogenboom *et al.* (1991) *Nuc. Acid Res.* 19:4133-4137;
Barbas *et al.* (1991) *Proc. Natl. Acad. Sci. USA*
88:7978-7982; and McCafferty *et al.* *Nature* (1990)
348:552-554.

25

[0140]   Additionally, recombinant anti-marker protein
antibodies, such as chimeric and humanized monoclonal
antibodies, comprising both human and non-human
portions, which can be made using standard recombinant
30 DNA techniques, are within the scope of the invention.
Such chimeric and humanized monoclonal antibodies can
be produced by recombinant DNA techniques known in the
art, for example using methods described in Robinson *et
al.* International Application No. PCT/US86/02269;

Akira, *et al.* European Patent Application 184,187;
Taniguchi, M., European Patent Application 171,496;
Morrison *et al.* European Patent Application 173,494;
Neuberger *et al.* PCT International Publication No.

5   WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567;
Cabilly *et al.* European Patent Application 125,023;
Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.*
(1987) *Proc. Natl. Acad Sci. USA* 84:3439-3443; Liu *et
al.* (1987) *J. Immunol.* 139:3521 3526; Sun et al. (1987)

10   *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura *et al.*
(1987) *Canc. Res.* 47:999-1005; Wood *et al.* (1985)
Nature 314:446-449; and Shaw *et al.* (1988) *J. Natl.
Cancer Inst.* 80:1553-1559); Morrison, S.L. (1985)
Science 229:1202-1207; Oi *et al.* (1986) *BioTechniques*

15   4:214; Winter U.S. Patent 5,225,539; Jones et al.
(1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988)
Science 239:1534; and Beidler *et al.* (1988) *J. Immunol.*
141:4053-4060.

20   **[0141]** Humanized antibodies are particularly desirable
for therapeutic treatment of human subjects. Humanized
forms of non-human (e.g. murine) antibodies are
chimeric molecules of immunoglobulins, immunoglobulin
chains or fragments thereof (such as Fv, Fab, Fab',

25   F(ab')$_2$ or other antigen-binding subsequences of
antibodies) which contain minimal sequence derived from
non-human immunoglobulin. Humanized antibodies include
human immunoglobulins (recipient antibody) in which
residues forming a complementary determining region

30   (CDR) of the recipient are replaced by residues from a
CDR of a non-human species (donor antibody) such as
mouse, rat or rabbit having the desired specificity,
affinity and capacity. In some instances, Fv framework
residues of the human immunoglobulin are replaced by

- 72 -

corresponding non-human residues. Humanized antibodies
may also comprise residues which are found neither in
the recipient antibody nor in the imported CDR or
framework sequences. In general, the humanized
5 antibody will comprise substantially all of at least
one, and typically two, variable domains, in which all
or substantially all of the CDR regions correspond to
those of a non-human immmunoglobulin and all or
substantially all of the constant regions being those
10 of a human immunoglobulin consensus sequence. The
humanized antibody will preferably also comprise at
least a portion of an immunoglobulin constant region
(Fc), typically that of a human immunoglobulin (Jones
et al. *Nature* 321: 522-525 (1986); Riechmann et al,
15 *Nature* 323: 323-329 (1988); and Presta
Curr.Op.Struct.Biol. 2: 594-596 (1992).

[0142] Such humanized antibodies can be produced using
transgenic mice which are incapable of expressing
20 endogenous immunoglobulin heavy and light chains genes,
but which can express human heavy and light chain
genes. The transgenic mice are immunized in the normal
fashion with a selected antigen, *e.g.*, all or a portion
of a polypeptide corresponding to a marker of the
25 invention. Monoclonal antibodies directed against the
antigen can be obtained using conventional hybridoma
technology. The human immunoglobulin transgenes
harbored by the transgenic mice rearrange during B cell
differentiation, and subsequently undergo class
30 switching and somatic mutation. Thus, using such a
technique, it is possible to produce therapeutically
useful IgG, IgA and IgE antibodies. For an overview of
this technology for producing humanized antibodies, see
Lonberg and Huszar (1995) *Int. Rev. Immunol.* 13:65-93).
35 For a detailed discussion of this technology for

- 73 -

producing humanized antibodies and humanized monoclonal
antibodies and protocols for producing such antibodies,
see, *e.g.*, U.S. Patent 5,625,126; U.S. Patent
5,633,425; U.S. Patent 5,569,825; U.S. Patent
5   5,661,016; and U.S. Patent 5,545,806.   In addition,
companies such as Abgenix, Inc. (Freemont, CA), can be
engaged to provide humanized antibodies directed
against a selected antigen using technology similar to
that described above.

10

[0143]   Humanized antibodies which recognize a selected
epitope can be generated using a technique referred to
as "guided selection."   In this approach a selected
non-human monoclonal antibody, *e.g.*, a murine antibody,
15   is used to guide the selection of a humanized antibody
recognizing the same epitope (Jespers *et al.*, 1994,
Bio/technology 12:899-903).

[0144]   An anti-marker protein antibody (*e.g.*,
20   monoclonal antibody) can be used to isolate a marker
protein of the invention by standard techniques, such
as affinity chromatography or immunoprecipitation.   An
anti-marker protein antibody can facilitate the
purification of natural marker proteins from cells and
25   of recombinantly produced marker proteins expressed in
host cells.   Moreover, an anti-marker protein antibody
can be used to detect marker protein (*e.g.*, in a
cellular lysate or cell supernatant) in order to
evaluate the abundance and pattern of expression of the
30   marker protein.   Anti-marker protein antibodies can be
used diagnostically to monitor protein levels in tissue
as part of a clinical testing procedure, *e.g.*, to, for
example, determine the efficacy of a given treatment
regimen.   Detection can be facilitated by coupling

- 74 -

(*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent
5   materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatasc, galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and
10 avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material
15 includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

20 III. Recombinant Expression Vectors and Host Cells

[0145]   Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding a marker protein of the invention
25 (or a portion thereof). As used herein, the term "vector" includes a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which includes a circular double stranded DNA loop into which
30 additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial
35 vectors having a bacterial origin of replication and

- 75 -

episomal mammalian vectors). Other vectors (e.g., non-
episomal mammalian vectors) are integrated into the
genome of a host cell upon introduction into the host
cell, and thereby are replicated along with the host
5   genome. Moreover, certain vectors are capable of
directing the expression of genes to which they are
operatively linked. Such vectors are referred to
herein as "expression vectors". In general, expression
vectors of utility in recombinant DNA techniques are
10 often in the form of plasmids. In the present
specification, "plasmid" and "vector" can be used
interchangeably as the plasmid is the most commonly
used form of vector. However, the invention is
intended to include such other forms of expression
15 vectors, such as viral vectors (e.g., replication
defective retroviruses, adenoviruses and adeno-
associated viruses), which serve equivalent functions.

[0146]   The recombinant expression vectors of the
20 invention comprise a nucleic acid of the invention in a
form suitable for expression of the nucleic acid in a
host cell, which means that the recombinant expression
vectors include one or more regulatory sequences,
selected on the basis of the host cells to be used for
25 expression, which is operatively linked to the nucleic
acid sequence to be expressed. Within a recombinant
expression vector, "operably linked" is intended to
mean that the nucleotide sequence of interest is linked
to the regulatory sequences) in a manner which allows
30 for expression of the nucleotide sequence (e.g., in an
in vitro transcription/translation system or in a host
cell when the vector is introduced into the host cell).
The term "regulatory sequence" is intended to include
promoters, enhancers and other expression control
35 elements (e.g., polyadenylation signals). Such

- 76 -

regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct

5   constitutive expression of a nucleotide sequence in many types of host cells and those which direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in

10 the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby

15 produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, marker proteins, mutant forms of marker proteins, fusion proteins, and the like).

20 [0147] The recombinant expression vectors of the invention can be designed for expression of marker proteins in prokaryotic or eukaryotic cells. For example, marker proteins can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus

25 expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed

30 and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

[0148] Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing

- 77 -

constitutive or inducible promoters directing the
expression of either fusion or non-fusion proteins.
Fusion vectors add a number of amino acids to a protein
encoded therein, usually to the amino terminus of the
5   recombinant protein. Such fusion vectors typically
serve three purposes: 1) to increase expression of
recombinant protein; 2) to increase the solubility of
the recombinant protein; and 3) to aid in the
purification of the recombinant protein by acting as a
10   ligand in affinity purification. Often, in fusion
expression vectors, a proteolytic cleavage site is
introduced at the junction of the fusion moiety and the
recombinant protein to enable separation of the
recombinant protein from the fusion moiety subsequent .
15   to purification of the fusion protein. Such enzymes,
and their cognate recognition sequences, include Factor
Xa, thrombin and enterokinase. Typical fusion
expression vectors include pGEX (Pharmacia Biotech Inc;
Smith, D,B. and Johnson, K.S. (1988) *Gene* 67:31-40),
20   pMAL (New England Biolabs, Beverly, MA) and pRITS
(Pharmacia, Piscataway, NJ) which fuse glutathione S
transferase (GST), maltose E binding protein, or
protein A, respectively, to the target recombinant
protein.
25

[0149]   Purified fusion proteins can be utilized in
marker activity assays, (*e.g.*, direct assays or
competitive assays described in detail below), or to
generate antibodies specific for marker proteins, for
30   example.

[0150]   Examples of suitable inducible non-fusion *E.
coli* expression vectors include pTrc (Hmann *et al.*,
(1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*,

- 78 -

Gene Expression Technology: Methods in Enzymology 185,
Academic Press, San Diego, California (1990) 60-89).
Target gene expression from the pTrc vector relies on
host RNA polymerase transcription from a hybrid trp-lac

5   fusion promoter. Target gene expression from the pET
11d vector relies on transcription from a T7 gn10-lac
fusion promoter mediated by a coexpressed viral RNA
polymerase (T7 gn1). This viral polymerase is supplied
by host strains BL21(DE3) or HMS174(DE3) from a

10 resident prophage harboring a T7 gn1 gene under the
transcriptional control of the lacUV 5 promoter.

[0151]   One strategy to maximize recombinant protein
expression in *E. coli* is to express the protein in a

15 host bacteria with an impaired capacity to
proteolytically cleave the recombinant protein
(Gottesman, S., *Gene Expression Technology: Methods in
Enzymology* 185, Academic Press, San Diego, California
(1990) 119-128). Another strategy is to alter the

20 nucleic acid sequence of the nucleic acid to be
inserted into an expression vector so that the
individual codons for each amino acid are those
preferentially utilized in *E. coli* (Wade *et al.*, (1992)
Nucleic Acids Res. 20:2111-2118). Such alteration of

25 nucleic acid sequences of the invention can be carried
out by standard DNA synthesis techniques.

[0152]   In another embodiment, the marker protein
expression vector is a yeast expression vector.

30 Examples of vectors for expression in yeast *S.*
cerevisiae include pYepSec1 (Baldari, *et al.*, (1987)
Embo J. 6:229-234), pMFa (Kurjan and Herskowitz, (1982)
Cell 30:933-943), pJRY88 (Schultz *et al.*, 21987) *Gene*

- 79 -

54:113-123), pYES2 (InVitrogen Corporation, San Diego, CA), and picZ (Invitrogen Corp, San Diego, CA).

[0153]   Alternatively, marker proteins of the invention
5 can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf 9 cells) include the pAc series (Smith et al. (1983) Mol. Cell Biol. 3:2156-2165) and the pVL series
10 (Lucklow and Summers (1989) Virology 170:31-39).

[0154]   In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian
15 expression vectors include pCDM8 (Seed, B. (1987) Nature 329:840) and pMT2PC (Kaufman et al. (1987) EMBO J. 6:187-I95). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example,
20 commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh, E. F., and Maniatis, T. Molecular
25 Cloning: A Laboratory Manual. 2nd, ed.. Cold Spring Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac
30 fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a

- 80 -

resident prophage harboring a T7 gn1 gene under the
transcriptional control of the lacUV 5 promoter.

[0155]   One strategy to maximize recombinant protein
5   expression in *E. coli* is to express the protein in a
host bacteria with an impaired capacity to
proteolytically cleave the recombinant protein
(Gottesman, S., *Gene Expression Technology: Methods in
Enzymology* 185, Academic Press, San Diego, California
10   (1990) 119-128).   Another strategy is to alter the
nucleic acid sequence of the nucleic acid to be
inserted into an expression vector so that the
individual codons for each amino acid are those
preferentially utilized in *E. coli* (Wada *et al.*, (1992)
15   *Nucleic Acids Res.* 20:2111-2118).   Such alteration of
nucleic acid sequences of the invention can be carried
out by standard DNA synthesis techniques.

[0156]   In another embodiment, the marker protein
20   expression vector is a yeast expression vector.
Examples of vectors for expression in yeast *S.
cerevisiae* include pYepSec1 (Baldari, *et al.*, (1987)
Embo J. 6:229-234), pMFa (Kurjan and Herskowitz, (1982)
Cell 30:933-943), pJRY88 (Schultz et al., (1987) *Gene*
25   54:113-123), pYES2 (Invitrogen Corporation, San Diego,
CA), and picZ (InVitrogen Corp, San Diego, CA).

[0157]   Alternatively, marker proteins of the invention
can be expressed in insect cells using baculovirus
30   expression vectors.   Baculovirus vectors available for
expression of proteins in cultured insect cells (*e.g.*,
Sf9 cells) include the pAc series (Smith *et al.* (1983)
Mol. Cell Biol. 3:2156-2165) and the pVL series
(Lucklow and Summers (1989) *Virology* 170:31-39).

- 81 -

[0158]　In yet another embodiment, a nucleic acid of
the invention is expressed in mammalian cells using a
mammalian expression vector.　Examples of mammalian
5　expression vectors include pCDM8 (Seed, B. (1987)
Nature 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO
J.* 6:187-195).　When used in mammalian cells, the
expression vector's control functions are often
provided by viral regulatory elements.　For example,
10　commonly used promoters are derived from polyoma,
Adenovirus 2, cytomegalovirus and Simian Virus 40.　For
other suitable expression systems for both prokaryotic
and eukaryotic cells see chapters 16 and 17 of
Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular
15　Cloning: A Laboratory Manual. 2nd, ed, Cold Spring
Harbor Laboratory*, Cold Spring Harbor Laboratory Press,
Cold Spring Harbor, NY, 1989.

[0159]　In another embodiment, the recombinant
20　mammalian expression vector is capable of directing
expression of the nucleic acid preferentially in a
particular cell type (*e.g.*, tissue-specific regulatory
elements are used to express the nucleic acid).
Tissue-specific regulatory elements are known in the
25　art.　Non-limiting examples of suitable tissue-specific
promoters include the albumin promoter (liver-specific;
Pinkert *et al.* (1987) *Genes Dev.* 1:268-277), lymphoid-
specific promoters (Calame and Eaton (1988) *Adv.
Immunol.* 43:235-275), in particular promoters of T cell
30　receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-
733) and immunoglobulins (Banerji *et al.* (1983) *Cell*
33:729-740; Queen and Baltimore (1983) *Cell* 33:741-
748), neuron-specific promoters (*e.g.*, the
neurofilament promoter, Byrne and R.aaddle (1989) *Proc.*

- 82 -

Nall. Acad Sci. USA 86:5473-5477), pancreas-specific
promoters (Edlund *et al.* (1985) *Science* 230:912-916),
and mammary gland-specific promoters (*e.g.*, milk whey
promoter, U.S. Patent No. 4,873,316 and European

5   Application Publication No. 264,166). Developmentally-
regulated promoters are also encompassed, for example
the marine hox promoters (Kessel and Grass (I990)
Science 249:374-379) and the α-fetoprotein promoter
(Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

10

[0160] The invention further provides a recombinant
expression vector comprising a DNA molecule of the
invention cloned into the expression vector in an
antisense orientation. That is, the DNA molecule is

15   operatively linked to a regulatory sequence in a manner
which allows for expression (by transcription of the
DNA molecule) of an RNA molecule which is antisense to
mRNA corresponding to a gene of the invention (*e.g.*, a
gene set forth in Tables 1-5 or 7-10). Regulatory

20   sequences operatively linked to a nucleic acid cloned
in the antisense orientation can be chosen which direct
the continuous expression of the antisense RNA molecule
in a variety of cell types, for instance viral
promoters and/or enhancers, or regulatory sequences can

25   be chosen which direct constitutive, tissue specific or
cell type specific expression of antisense RNA. The
antisense expression vector can be in the form of a
recombinant plasmid, phagemid or attenuated virus in
which antisense nucleic acids are produced under the

30   control of a high efficiency regulatory region, the
activity of which can be determined by the cell type
into which the vector is introduced. For a discussion
of the regulation of gene expression using antisense
genes see Weintraub, H. *et al.*, Antisense RNA as a

- 83 -

molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1)1986.

[0161] Another aspect of the invention pertains to
5   host cells into which a nucleic acid molecule of the invention is introduced, *e.g.*, a gene set forth in Tables 1-5 or 7-10 within a recombinant expression vector or a nucleic acid molecule of the invention containing sequences which allow it to homologously
10   recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential
15   progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used
20   herein.

[0162] A host cell can be any prokaryotic or eukaryotic cell. For example, a marker protein of the invention can be expressed in bacterial cells such as
25   *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

30   [0163] Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for

- 84 -

introducing foreign nucleic acid (*e.g.*, DNA) into a
host cell, including calcium phosphate or calcium
chloride co-precipitation, DEAE-dextran-mediated
transfection, lipofection, or electmporation. Suitable
5   methods for transforming or transferring host cells can
be found in Sambrook, *et al.* (*Molecular Cloning: A
Laboratory Manual. 2nd, ed., Cold Spring Harbor
Laboratory*, Cold Spring Harbor Laboratory Press, Cold
Spring Harbor, NY,1989), and other laboratory manuals.
10

[0164] For stable transfection of mammalian cells, it
is known that, depending upon the expression vector and
transfection technique used, only a small fraction of
cells may integrate the foreign DNA into their genome.
15 In order to identify and select these integrants, a
gene that encodes a selectable flag (*e.g.*, resistance
to antibiotics) is generally introduced into the host
cells along with the gene of interest. Preferred
selectable flags include those which confer resistance
20 to drugs, such as G418, hygromycin and methotrexate.
Nucleic acid encoding a selectable flag can be
introduced into a host cell on the same vector as that
encoding a marker protein or can be introduced on a
separate vector. Cells stably transfected with the
25 introduced nucleic acid can be identified by drug
selection (*e.g.*, cells that have incorporated the
selectable flag gene will survive, while the other
cells die).

30 [0165] A host cell of the invention, such as a
prokaryotic or eukaryotic host cell in culture, can be
used to produce (*i.e.*, express) a marker protein.
Accordingly, the invention further provides methods for
producing a marker protein using the host cells of the

- 85 -

invention. In one embodiment, the method comprises
culturing the host cell of invention (into which a
recombinant expression vector encoding a marker protein
has been introduced) in a suitable medium such that a
5   marker protein of the invention is produced. In
another embodiment, the method further comprises
isolating a marker protein from the medium or the host
cell.

10   [0166] The host cells of the invention can also be
used to produce non-human transgenic animals. For
example, in one embodiment, a host cell of the
invention is a fertilized oocyte or an embryonic stem
cell into which marker-protein-coding sequences have
15   been introduced. Such host cells can then be used to
create non-human transgenic animals in which exogenous
sequences encoding a marker protein of the invention
have been introduced into their genome or homologous
recombinant animals in which endogenous sequences
20   encoding the marker proteins of the invention have been
altered. Such animals are useful for studying the
function and/or activity of a marker protein and for
identifying and/or evaluating modulators of marker
protein activity. As used herein, a "transgenic
25   animal" is a non-human animal, preferably a mammal,
more preferably a rodent such as a rat or mouse, in
which one or more of the cells of the animal includes a
transgene. Other examples of transgenic animals
include non-human primates, sheep, dogs, cows, goats,
30   chickens, amphibians, and the tike. A transgene is
exogenous DNA which is integrated into the genome of a
cell from which a transgenic animal develops and which
remains in the genome of the mature animal, thereby
directing the expression of an encoded gene product in
35   one or more cell types or tissues of the transgenic

- 86 -

animal. As used herein, a "homologous recombinant
animal" is a non-human animal, preferably a mammal,
more preferably a mouse, in which an endogenous gene of
the invention (e.g., a gene set forth in Tables 1-5 or
5   7-10) has been altered by homologous recombination
between the endogenous gene and an exogenous DNA
molecule introduced into a cell of the animal, e.g., an
embryonic cell of the animal, prior to development of
the animal.

10

[0167] A transgenic animal of the invention can be
created by introducing a marker-encoding nucleic acid
into the mate pronuclei of a fertilized oocyte, e.g.,
by microinjection, retroviral infection, and allowing
15   the oocyte to develop in a pseudopregnant female foster
animal. Intronic sequences and polyadenylation signals
can also be included in the transgene to increase the
efficiency of expression of the transgene. A tissue-
specific regulatory sequence(s) can be operably linked
20   to a transgene to direct expression of a marker protein
to particular cells. Methods for generating transgenic
animals via embryo manipulation and microinjection,
particularly animals such as mice, have become
conventional in the art and are described, for example,
25   in U.S. Patent Nos. 4,736,866 and 4,870,009, both by
Leder et al., U.S. Patent No. 4,873,191 by Wagner et
al. and in Hogan, B., Manipulating the Mouse Embryo,
(Cold Spring Harbor Laboratory Press, Cold Spring
Harbor, N.Y., 1986). Similar methods are used for
30   production of other transgenic animals. A transgenic
founder animal can be identified based upon the
presence of a transgene of the invention in its genome
and/or expression of mRNA corresponding to a gene of
the invention in tissues or cells of the animals. A
35   transgenic founder animal can then be used to breed

- 87 -

additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a marker protein can further be bred to other transgenic animals carrying other transgenes.

5

[0168] To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a gene of the invention into which a deletion, addition or substitution has been introduced to thereby alter,

10 e.g., functionally disrupt, the gene. The gene can be a human gene, but more preferably, is a non-human homologue of a human gene of the invention (e.g., a gene set forth in Tables 1-5 or 7-10). For example, a mouse gene can be used to construct a homologous

15 recombination nucleic acid molecule, e.g., a vector, suitable far altering an endogenous gene of the invention in the mouse genome. In a preferred embodiment, the homologous recombination nucleic acid molecule is designed such that, upon homologous

20 recombination, the endogenous gene of the invention is functionally disrupted (i.e., no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the homologous recombination nucleic acid molecule can be designed such that, upon

25 homologous recombination, the endogenous gene is mutated or otherwise altered but still encodes functional protein (e.g., the upstream regulatory region can be altered to thereby alter the expression of the endogenous marker protein). In the homologous

30 recombination nucleic acid molecule, the altered portion of the gene of the invention is flanked at its 5' and 3' ends by additional nucleic acid sequence of the gene of the invention to allow for homologous recombination to occur between the exogenous gene

35 carried by the homologous recombination nucleic acid

- 88 -

molecule and an endogenous gene in a cell, *e.g.*, an
embryonic stem cell. The additional flanking nucleic
acid sequence is of sufficient length for successful
homologous recombination with the endogenous gene.

5   Typically, several kilobases of flanking DNA (both at
the 5' and 3' ends) are included in the homologous
recombination nucleic acid molecule (see, *e.g.*, Thomas,
K.R. and Capecchi, M.R. (1987) *Cell* 51:503 for a
description of homologous recombination vectors). The

10   homologous recombination nucleic acid molecule is
introduced into a cell, *e.g.*, an embryonic stem cell
line (*e.g.*, by electroporation) and cells in which the
introduced gene has homologously recombined with the
endogenous gene are selected (see *e.g.*, Li, E. *et al.*

15   (1992) *Cell* 69:915). The selected cells can then
injected into a blastocyst of an animal (*e.g.*, a mouse)
to form aggregation chimeras (see *e.g.* Bradley, S A. in
*Teratocareirtomas and Embryonic Stem Cells: A
Practical Approach*, E.J. Robertson, ed. (IRL, Oxford,

20   1987) pp. 113-152). A chimeric embryo can then be
implanted into a suitable pseudopregnant female foster
animal and the embryo brought to term. Progeny
harboring the homologously recombined DNA in their germ
cells can be used to breed animals in which all cells

25   of the animal contain the homologously recombined DNA
by germline transmission of the transgene. Methods for
constructing homologous recombination nucleic acid
molecules, *e.g.*, vectors, or homologous recombinant
animals are described further in Bradley, A. (1991)

30   *Current Opinion in Biotechnology* 2:823-829 and in PCT
International Publication Nos.: WO 90/11354 by Le
Mouellec *et al.*; WO 91/01140 by Smithies *et al.*; WO
92/0968 by Zijlstra *et al.*; and WO 93/04169 by Berns *et
al.*

- 89 -

[0169]   In another embodiment, transgenic non-human
animals can be produced which contain selected systems
which allow for regulated expression of the transgene.
One example of such a system is the *cre/loxP*
recombinase system of bacteriophage P1. For a
description of the *cre/loxP* recombinase system, see,
e.g., Laksa *et al.* (1992) *Proc. Natl. Acad. Sci. USA*
89:6232-6236. Another example of a recombinase system
is the FLP recombinase system of *Saccharomyces
cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-
1355. If a *cre/loxP* recombinase system is used to
regulate expression of the transgene, animals
containing transgenes encoding both the *Cre* recombinase
and a selected protein are required. Such animals can
be provided through the construction of "double"
transgenic animals, *e.g.*, by mating two transgenic
animals, one containing a transgene encoding a selected
protein and the other containing a transgene encoding a
recombinase.

[0170]   Clones of the non-human transgenic animals
described herein can also be produced according to the
methods described in Wilmut, I. *et al.* (1997) *Nature*
385:810-813 and PCT International Publication Nos. WO
97/07668 and WO 97/07669. In brief, a cell, *e.g.*, a
somatic cell, from the transgenic animal can be
isolated and induced to exit the growth cycle and enter
G_0 phase. The quiescent cell can then be fused, *e.g.*,
through the use of electrical pulses, to an enucleated
oocyte from an animal of the same species from which
the quiescent cell is isolated. The reconstructed
oocyte is then cultured such that it develops to morula
or blastocyte and then transferred to pseudopregnant

- 90 -

female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is isolated.

5   IV.   Pharmaceutical Compositions

[0171]   The nucleic acid molecules of the invention (*e.g.*, the genes set forth in Tables 1-5 or 7-10), fragments of marker proteins, and anti-marker protein
10   antibodies of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions (also referred to herein as "bioactive agents or compounds") typically comprise the nucleic acid molecule, protein, or
15   antibody and a pharmaceutically acceptable carrier.

[0172]   As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and
20   antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well-known in the art. Except insofar as any conventional media or agent
25   is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary bioactive agents can also be incorporated into the compositions.

30   [0173]   The invention includes methods for preparing pharmaceutical compositions for modulating the expression or activity of a polypeptide or nucleic acid corresponding to a marker of the invention. Such methods comprise formulating a pharmaceutically

- 91 -

acceptable carrier with an agent which modulates
expression or activity of a polypeptide or nucleic acid
corresponding to a marker of the invention.   Such
compositions can further include additional active
5   agents.   Thus, the invention further includes methods
for preparing a pharmaceutical composition by
formulating a pharmaceutically acceptable carrier with
an agent which modulates expression or activity of a
polypeptide or nucleic acid corresponding to a marker
10   of the invention and one or more additional bioactive
agents.

[0174]   The invention also provides methods (also
referred to herein as "screening assays") for
15   identifying modulators, i.e., candidate or test
compounds or agents comprising therapeutic moieties
(e.g., peptides, peptidomimetics, peptoids, small
molecules or other drugs) which (a) bind to the marker,
or (b) have a modulatory (e.g., stimulatory or
20   inhibitory) effect on the activity of the marker or,
more specifically, (c) have a modulatory effect on the
interactions of the marker with one or more of its
natural substrates (e.g., peptide, protein, hormone,
co-factor, or nucleic acid), or (d) have a modulatory
25   effect on the expression of the marker.   Such assays
typically comprise a reaction between the marker and
one or more assay components.   The other components may
be either the test compound itself, or a combination of
test compound and a natural binding partner of the
30   marker.

[0175]   The test compounds of the present invention may
be bioactive agents, i.e. protein, oligopeptide,
molecule, polysaccharide, polynucleotides.   In a
35   preferred embodiment the bioactive agents are proteins,

- 92 -

in particular naturally occurring proteins or fragments thereof.

[0176] The test compounds of the present invention may
5   be obtained from any available source, including
systematic libraries of natural and/or synthetic
compounds. Test compounds may also be obtained by any
of the numerous approaches in combinatorial library
methods known in the art, including: biological
10 libraries; peptoid libraries (libraries of molecules
having the functionalities of peptides, but with a
novel, non-peptide backbone which are resistant to
enzymatic degradation but which nevertheless remain
bioactive; see, e.g., Zuckermann et al., 1994, J. Med.
15 Chem. 37:2678-85); spatially addressable parallel solid
phase or solution phase libraries; synthetic library
methods requiring deconvolution; the 'one-bead one-
compound' library method; and synthetic library methods
using affinity chromatography selection. The
20 biological library and peptoid library approaches are
limited to peptide libraries, while the other four
approaches are applicable to peptide, non-peptide
oligomer or small molecule libraries of compounds (Lam,
1997, Anticancer Drug Des. 12:145).
25

[0177] A pharmaceutical composition of the invention
is formulated to be compatible with its intended route
of administration. Examples of routes of
administration include parenteral, e.g., intravenous,
30 intradermal, subcutaneous, oral (e.g., inhalation),
transdermal (topical), transmucosal, and rectal
administration. Solutions or suspensions used for
parenteral, intradermal, or subcutaneous application
can include the following components: a sterile

- 93 -

diluent such as water for injection, saline solution,
fixed oils, polyethylene glycols, glycerine; propylene
glycol or other synthetic solvents; antibacterial
agents such as benzyl alcohol or methyl parabens;
5   antioxidants such as ascorbic acid or sodium bisulfate;
chelating agents such as ethylenediaminetetraacetic
acid; buffers such as acetates, citrates or phosphates
and agents for the adjustment of tonicity such as
sodium chloride or dextrose. pH can be adjusted with
10 acids or bases, such as hydrochloric acid or sodium
hydroxide. The parenteral preparation can be enclosed
in ampoules, disposable syringes or multiple dose vials
made of glass or plastic.

15 [0178] Pharmaceutical compositions suitable for
injectable use include sterile aqueous solutions (where
water soluble) or dispersions and sterile powders for
the extemporaneous preparation of sterile injectable
solutions or dispersion. For intravenous
20 administration, suitable carriers include physiological
saline, bacteriostatic water, Cremophor EL™ (BASF,
Parsippany, NJ) or phosphate buffered saline (PBS). In
all cases, the composition must be sterile and should
be fluid to the extent that easy syringability exists.
25 It must be stable under the conditions of manufacture
and storage and must be preserved against the
contaminating action of microorganisms such as bacteria
and fungi. The earner can be a solvent or dispersion
medium containing, for example, water, ethanol, polyol
30 (for example, glycerol, propylene glycol, and liquid
polyetheylene glycol, and the like), and suitable
mixtures thereof. The proper fluidity can be
maintained, for example, by the use of a coating such
as lecithin, by the maintenance of the requited
35 particle size in the case of dispersion and by the use

- 94 -

of surfactants. Prevention of the action of
microorganisms can be achieved by various antibacterial
and antifungal agents, for example, parabens,
chlorobutanol, phenol, ascorbic acid, thimerosal, and
5   the like. In many cases, it will be preferable to
include isotonic agents, for example, sugars,
polyalcohols such as manitol, sorbitol, sodium chloride
in the composition. Prolonged absorption of the
injectable compositions can be brought about by
10   including in the composition an agent which delays
absorption, for example, aluminum monostearate and
gelatin.

[0179] Sterile injectable solutions can be prepared by
15   incorporating the active compound (e.g., a fragment of
a marker protein or an anti-marker protein antibody) in
the required amount in an appropriate solvent with one
or a combination of ingredients enmnerated above, as
required, followed by filtered sterilization.
20   .Generally, dispersions are prepared by incorporating
the active compound into a sterile vehicle which
contains a basic dispersion medium and the required
other ingredients from those enumerated above. In the
case of sterile powders for the preparation of sterile
25   injectable solutions, the preferred methods of
preparation are vacuum drying and freeze-drying which
yields a powder of the active, ingredient plus any
additional desired ingredient from a previously
sterile-filtered solution thereof.
30

[0180] Oral compositions generally include an inert
diluent or an edible carrier. They can be enclosed in
gelatin capsules or compressed into tablets. For the
purpose of oral therapeutic administration, the active
35   compound can be incorporated with excipients and used

- 95 -

in the form of tablets, troches, or capsules. Oral
compositions can also be prepared using a fluid carrier
for use as a mouthwash, wherein the compound in the
fluid carrier is applied orally and swished and

5   expectorated or swallowed. Pharmaceutically compatible
binding agents, and/or adjuvant materials can be
included as part of the composition. The tablets,
pills, capsules, troches and the like can contain any
of the following ingredients, or compounds of a similar

10   nature: a binder such as microcrystalline cellulose,
gum tragacanth or gelatin; an excipient such as starch
or lactose, a disintegrating agent such as alginic
acid, Primogel, or corn starch; a lubricant such as
magnesium stearate or Stertes; a glidant such as

15   colloidal silicon dioxide; a sweetening agent such as
sucrose or saccharin; or a flavoring agent such as
peppermint, methyl salicylate, or orange flavoring.

[0181] For administration by inhalation, the compounds

20   are delivered in the form of an aerosol spray from
pressured container or dispenser which contains a
suitable propellant, e.g., a gas such as carbon
dioxide, or a nebulizer.

25   [0182] Systemic administration can also be by
transmucosal or transdermal means. For transmucosal or
transdermal administration, penetrants appropriate to
the barrier to be permeated are used in the
formulation. Such penetrants are generally known in

30   the art, and include, for example, for transmucosal
administration, detergents, bile salts, and fusidic
acid derivatives. Transmucosal administration can be
accomplished through the use of nasal sprays or
suppositories. For transdermal administration, the

- 96 -

bioactive compounds are formulated into ointments,
salves, gels, or creams as generally known in the art.

[0183]   The compounds can also be prepared in the form
5   of suppositories (e.g., with conventional suppository
bases such as cocoa butter and other glycerides) or
retention enemas for rectal delivery.

[0184]   In one embodiment, the therapeutic moieties,
10   which may contain a bioactive compound, are prepared
with carriers that will protect the compound against
rapid elimination from the body, such as a controlled
release formulation, including implants and
microencapsulated delivery systems. Biodegradable,
15   biocompatible polymers can be used, such as ethylene
vinyl acetate, polyanhydrides, polyglycolic acid,
collagen, polyorthoesters, and polylactic acid.
Methods for preparation of such formulations will be
apparent to those skilled in the art. The materials
20   can also be obtained commercially from Alza Corporation
and Nova Pharmaceuticals, Inc. Liposomal suspensions
(including liposomes targeted to infected cells with
monoclonal antibodies to viral antigens) can also be
used as pharmaceutically acceptable carriers. These
25   can be prepared according to methods known to those
skilled in the art, for example, as described in U.S.
Patent No. 4,522,811.

[0185]   It is especially advantageous to formulate oral
30   or parenteral compositions in dosage unit form for ease
of administration and uniformity of dosage. Dosage
unit form as used herein includes physically discrete
units suited as unitary dosages for the subject to be
treated; each unit containing a predetermined quantity

- 97 -

of active compound calculated to produce the desired
therapeutic effect in association with the required
pharmaceutical carrier. The specification for the
dosage unit forms of the invention are dictated by and
5   directly dependent on-the unique characteristics of the
active compound and the particular therapeutic effect
to be achieved, and the limitations inherent in the art
of compounding such an active compound for the
treatment of individuals.

10

[0186]   Toxicity and therapeutic efficacy of such
compounds can be determined by standard pharmaceutical
procedures in cell cultures or experimental animals,
e.g., for determining the LD50 (the dose lethal to 50%
15   of the population) and the ED50 (the dose
therapeutically effective in 50% of the population).
The dose ratio between toxic and therapeutic effects is
the therapeutic index and it can be expressed as the
ratio LD50/ED50. Compounds which exhibit large
20   therapeutic indices are preferred. While compounds
that exhibit toxic side effects may be used, care
should be taken to design a delivery system that
targets such compounds to the site of affected tissue
in order to minimize potential damage to uninfected
25   cells and, thereby, reduce side effects.

[0187]   The data obtained from the cell culture assays
and animal studies can be used in formulating a range
of dosage for use in humans. The dosage of such
30   compounds lies preferably within a range of circulating
concentrations that include the ED50 with little or no
toxicity. The dosage may vary within this range
depending upon the dosage form employed and the route
of administration utilized. For any compound used in
35   the method of the invention, the therapeutically

- 98 -

effective dose can be estimated initially from cell
culture assays. A dose may be formulated in animal
models to achieve a circulating plasma concentration
range that includes the IC50 (*i.e.*, the concentration
5   of the test compound which achieves a half-maximal
inhibition of symptoms) as determined in cell culture.
Such information can be used to more accurately
determine useful doses in humans. Levels in plasma may
be measured, for example, by high performance liquid
10 chromatography.

[0188] The nucleic acid molecules of the invention can
be inserted into vectors and used as gene therapy
vectors. Gene therapy vectors can be delivered to a
15 subject by, for example, intravenous injection, local
administration (see U.S. Patent 5,328,470) or by
stereotactic injection (see *e.g.*, Chen *et al.* (1994)
Proc. Natl. Acad. Sci. USA 91:3054-3057). The
pharmaceutical preparation of the gene therapy vector
20 can include the gene therapy vector in an acceptable
diluent, or can comprise a slow release matrix in which
the gene delivery vehicle is imbedded. Alternatively,
where the complete gene delivery vector can be produced
intact from recombinant cells, *e.g.*, retroviral
25 vectors, the pharmaceutical preparation can include one
or more cells which produce the gene delivery system.

[0189] The pharmaceutical compositions can be included
in a container, pack, or dispenser together with
30 instructions for administration.

- 99 -

V.   Computer Readable Means and Arrays

[0190]   Computer readable media comprising a marker(s)
of the present invention is also provided. As used
5   herein, "computer readable media" includes a medium
that can be read and accessed directly by a computer.
Such media include, but are not limited to: magnetic
storage media, such as floppy discs, hard disc storage
medium, and magnetic tape; optical storage media such
10   as CD-ROM; electrical storage media such as RAM and
ROM; and hybrids of these categories such as
magnetic/optical storage media. The skilled artisan
will readily appreciate how any of the presently known
computer readable mediums can be used to create a
15   manufacture comprising computer readable medium having
recorded thereon a marker of the present invention.

[0191]   As used herein, "recorded" includes a process
for storing information on computer readable medium.
20   Those skilled in the art can readily adopt any of the
presently known methods for recording information on
computer readable medium to generate manufactures
comprising the markers of the present invention.

25   [0192]   A variety of data processor programs and
formats can be used to store the marker information of
the present invention on computer readable medium. For
example, the nucleic acid sequence corresponding to the
markers can be represented in a word processing text
30   file, formatted in commercially-available software such
as WordPerfect and Microsoft Word, or represented in
the form of an ASCII file, stored in a database
application, such as DB2, Sybase, Oracle, or the like.
Any number of dataprocessor structuring formats (e.g.,

- 100 -

text file or database) may be adapted in order to
obtain computer readable medium having recorded thereon
the markers of the present invention.

5   [0193]   By providing the markers of the invention in
computer readable form, one can routinely access the
marker sequence information for a variety of purposes.
For example, one skilled in the art can use the
nucleotide or amino acid sequences of the invention in
10 computer readable form to compare a target sequence or
target structural motif with the sequence information
stored within the data storage means.   Search means are
used to identify fragments or regions of the sequences
of the invention which match a particular target
15 sequence or target motif.

[0194]   The invention also includes an array comprising
a marker(s) of the present invention, i.e. a biochip.
The array can be used to assay expression of one or
20 more genes in the array.   In one embodiment, the array
can be used to assay gene expression in a tissue to
ascertain tissue specificity of genes in the array.   In
this manner, up to about 8600 genes can be
simultaneously assayed for expression.   This allows an
25 expression profile to be developed showing a battery of
genes specifically expressed in one or more tissues at
a given point in time.

[0195]   In addition to such qualitative determination,
30 the invention allows the quantitation of gene
expression in the biochip.   Thus, not only tissue
specificity, but also the level of expression of a
battery of genes in the tissue is ascertainable.   Thus,
genes can be grouped on the basis of their tissue
35 expression per se and level of expression in that

- 101 -

tissue. As used herein, a "normal level of expression"
refers to the level of expression of a gene provided in
a control sample, typically the control is from non-
involved cells or tissues, or from a non-diseased

5   subject. The determination of normal levels of
expression is useful, for example, in ascertaining the
relationship of gene expression between or among
tissues. Thus, one tissue can be perturbed and the
effect on gene expression in a second tissue can be

10   determined. In this context, the effect of one cell
type on another cell type in response to a biological
stimulus can be determined. Such a determination is
useful, for example, to know the effect of cell-cell
interaction at the level of gene expression. If an

15   agent is administered therapeutically to treat one cell
type but has an undesirable effect on another cell
type, the invention provides an assay to determine the
molecular basis of the undesirable effect and thus
provides the opportunity to co-administer a

20   counteracting agent or otherwise treat the undesired
effect. Similarly, even within a single cell type,
undesirable biological effects can be determined at the
molecular level. Thus, the effects of an agent on
expression of other than the target gene can be

25   ascertained and counteracted.

[0196]   In another embodiment, the arrays can be used
to monitor the time course of expression of one or more
genes in the array. This can occur in various

30   biological contexts, as disclosed herein, for example
development and differentiation, disease progression,
in vitro processes, such a cellular transformation and
senescence, autonomic neural and neurological
processes, such as, for example, pain and appetite, and

35   cognitive functions, such as learning or memory.

[0197] The array is also useful for ascertaining the effect of the expression of a gene on the expression of other genes in the same cell or in different cells.
5 This provides, for example, for a selection of alternate molecular targets for therapeutic intervention if the ultimate or downstream target cannot be regulated.

10 [0198] The array is also useful for ascertaining differential expression patterns of one or more genes in non-involved or diseased cells. This provides a battery of genes that could serve as a molecular target for diagnosis or therapeutic intervention. In
15 particular, biochips can be made comprising arrays not only of the differentially expressed markers listed in Tables 1-5 or 7-10, but of markers specific to subjects at a certain stage of the disease (i.e. secondary progressive, primary progressive, relapsing-remitting),
20 or from higher-risk geographical regions (i.e. northern Europe or Canada).

VI. Predictive Medicine

25 [0199] The present invention pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenetics and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual
30 prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining marker protein and/or nucleic acid expression as welt as marker protein activity, in the context of a biological sample (e.g., blood, serum,

- 103 -

cells, tissue) to thereby determine whether an
individual is afflicted with a disease or disorder, or
is at risk of developing a disorder, associated with
increased or decreased marker protein expression or
5   activity. The invention also provides for prognostic
(or predictive) assays for determining whether an
individual is at risk of developing a disorder
associated with marker protein, nucleic acid expression
or activity. For example, the number of copies of a
10   marker gene can be assayed in a biological sample.
Such assays can be used for prognostic or predictive
purposes to thereby phophylactically treat an
individual prior to the onset of a disorder (e.g.,
multiple sclerosis)characterized by or associated with
15   marker protein, nucleic acid expression or activity.

[0200] Another aspect of the invention pertains to
monitoring the influence of agents (e.g., drugs,
compounds) on the expression or activity of marker in
20   clinical trials.

[0201] These and other agents are described in further
detail in the following sections.

25   1.   Diagnostic Assays

[0202] An exemplary method for detecting the presence
or absence of marker protein or nucleic acid of the
invention in a biological sample involves obtaining a
30   biological sample from a test subject and contacting
the biological sample with a compound or an agent
capable of detecting the protein or nucleic acid (e.g.,
mRNA, genomic DNA) that encodes the marker protein such
that the presence of the marker protein or nucleic acid

- 104 -

is detected in the biological sample. A preferred
agent for detecting mRNA or genomic DNA corresponding
to a marker gene or protein of the invention is a
labeled nucleic acid probe capable of hybridizing to a
5   mRNA or genomic DNA of the invention. Suitable probes
for use in the diagnostic assays of the invention are
described herein.

[0203] A preferred agent for detecting marker protein
10   is an antibody capable of binding to marker protein,
preferably an antibody with a detectable label.
Antibodies can be polyclonal, or more preferably,
monoclonal. An intact antibody, or a fragment thereof
(e.g., Fab or F(ab')$_2$) can be used. The term "labeled",
15   with regard to the probe or antibody, is intended to
encompass direct labeling of the probe or antibody by
coupling (i.e., physically linking) a detectable
substance to the probe or antibody, as well as indirect
labeling of the probe or antibody by reactivity with
20   another reagent that is directly labeled. Examples of
indirect labeling include detection of a primary
antibody using a fluorescently labeled secondary
antibody and end-labeling of a DNA probe with biotin
such that it can be detected with fluorescently labeled
25   streptavidin. The term "biological sample" is intended
to include tissues, cells and biological fluids
isolated from a subject, as well as tissues, cells and
fluids present within a subject. That is, the
detection method of the invention can be used to defeat
30   marker mRNA, protein, or genomic DNA in a biological
sample in vitro as well as in vivo. For example, in
vitro techniques for detection of marker mRNA include
Northern hybridizations and in situ hybridizations. In
vitro techniques for detection of marker protein
35   include enzyme linked immunosorbent assays (ELISAs),

- 105 -

Western blots, immunoprecipitations and
immunofluoresCence. *In vitro* techniques for detection
of marker genomic DNA include Southern hybridizations.
Furthermore, *in vivo* techniques for detection of marker
5   protein include introducing into a subject a labeled
anti-marker antibody. For example, the antibody can be
labeled with a radioactive marker whose presence and
location in a subject can be detected by standard
imaging techniques.
10

[0204] In one embodiment, the biological sample
contains protein molecules from the test subject.
Alternatively, the biological sample can contain mRNA
molecules from the test subject or genomic DNA
15   molecules from the test subject. A preferred
biological sample is a serum sample isolated by
conventional means from a subject.

[0205] In another embodiment, the methods further
20   involve obtaining a control biological sample (*e.g.*,
noninvolved tissue or from a non-diseased subject) from
a control subject, contacting the control sample with a
compound or agent capable of detecting marker protein,
mRNA, or genomic DNA, such that the presence of marker
25   protein, mRNA or genomic DNA is detected in the
biological sample, and comparing the presence of marker
protein, mRNA or genomic DNA in the control sample with
the presence of marker protein, mRNA or genomic DNA in
the test sample.
30

[0206] The invention also encompasses kits for
detecting the presence of marker in a biological
sample. For example, the kit can comprise a labeled
compound or agent capable of detecting marker protein

- 106 -

or mRNA in a biological sample; means for determining
the amount of marker in the sample; and means for
comparing the amount of marker in the sample with a
standard. The compound or agent can be packaged in a
5   suitable container. The kit can further comprise
instructions for using the kit to detect marker protein
or nucleic acid.

          2.   Prognostic Assays
10

     [0207]   The diagnostic methods, described herein can
     furthermore be utilized to identify subjects having or
     at risk of developing a disease or disorder associated
     with aberrant marker expression or activity. As used
15   herein, the term "aberrant" includes a marker
     expression or activity which deviates from the wild
     type marker expression or activity. Aberrant
     expression or activity includes increases or decreased
     expression or activity, as well as expression or
20   activity which does not follow the wild type
     developmental pattern of expression or the subcellular
     pattern of expression. For example, aberrant marker
     expression or activity is intended to include the cases
     in which a mutation in the marker ·gene causes the
25   marker gene to be under-expressed or over-expressed and
     situations in which such mutations result in a non-
     functional marker protein or a protein which does not
     function in a wild-type fashion, e.g., a protein which
     does not interact with a marker ligand or one which
30   interacts with a non marker protein ligand.

     [0208]   The assays described herein, such as the
     preceding diagnostic assays or the following assays,
     can be utilized to identify a subject having or at risk
35   of developing a disorder associated with a

- 107 -

misregulation in marker protein activity or nucleic
acid expression, such as multiple sclerosis.
Alternatively, the prognostic assays can be utilized to
identify a subject having or at risk for developing a,
5   disorder associated with a misregulation in marker
·protein activity or nucleic acid expression, such as
multiple sclerosis. Thus, the present invention
provides a method for identifying a disease or disorder
associated with aberrant marker expression or activity
10 in which a test sample is obtained from a subject and
marker protein or nucleic acid (e.g., mRNA or genomie
DNA) is detected, wherein the presence of marker
protein or nucleic acid is diagnostic for a subject
having or at risk of developing a disease or disorder
15 associated with aberrant marker expression or activity.
As used herein, a "test sample" includes a biological
sample obtained from a subject of interest. For
example, a test sample can be a biological fluid (e.g.,
blood PBMCs), cell sample, or tissue (e.g., brain).

20

[0209] Furthermore, the prognostic assays described·
herein can be used to determine whether a subject can
be administered an agent (e.g., an agonist, antagonist,
peptidomimetic, protein, peptide, nucleic acid, small
25 molecule, or other drug candidate) to treat a disease
or disorder associated with increased or degreased
marker expression or activity. For example, such
methods can be used to determine whether a subject can
be effectively treated with an agent for a disorder
30 such as multiple sclerosis. Thus, the present
invention provides methods for determining whether a
subject can be effectively treated with an agent for a
disorder associated with increased or decreased marker
expression or activity in which a test sample is
35 obtained and marker protein or nucleic acid expression

- 108 -

or activity is detected (*e.g.*, wherein the abundance of
marker protein or nucleic acid expression or activity
is diagnostic for a subject that can be administered
the agent to treat a disorder associated with increased
5   or decreased marker expression or activity).

[0210] The methods of the invention can also be used
to detect genetic alterations in a marker gene, thereby
determining if a subject with the altered gene is at
10   risk for a disorder characterized by misregulation in
marker protein activity or nucleic acid expression,
such as multiple sclerosis. In preferred embodiments,
the methods include detecting, in a sample of cells
from the subject, the presence or absence of a genetic
15   alteration characterized by at least one of an
alteration affecting the integrity of a gene encoding a
marker-protein, or the mis-expression of the marker
gene. For example, such genetic alterations can be
detected by ascertaining the existence of at least one
20   of 1) a deletion of one or more nucleotides from a
marker gene; 2) an addition of one or more nucleotides
to a marker gene; 3) a substitution of one or more
nucleotides of a marker gene, 4) a chromosomal
rearrangement of a marker gene; 5) an alteration in the
25   level of a messenger RNA transcript of a marker gene,
6) aberrant modification of a ma=ker gene, such as of
the methylation pattern of the genomic DNA, 7) the
presence of a non-wild type splicing pattern of a
messenger RNA transcript of a marker gene, 8) a non-
30   wild type level of a marker-protein, 9) allelic loss of
a marker gene, and 10) inappropriate post-translational
modification of a marker-protein. As described herein,
there are a large number of assays known in the art
which can be used for detecting alterations in a marker
35   gene. A preferred biological sample is a tissue (*e.g.*,

- 109 -

brain) or blood sample isolated by conventional means from a subject.

[0211]   In certain embodiments, detection of the alteration involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, e.g., U.S. Patent Nos. 4,683,(95 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, e.g., Landegran et al. (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *Proc. Mail. Acad. Sci. USA* 91:360-364), the latter of which can be particularly useful for detecting point mutations in the marker-gene (see Abravaya *et al.* (1995) *Nucleic Acids Res.* 23:675-682). This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a marker gene under conditions such that hybridization and amplification of the marker-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

[0212]   Alternative amplification methods include: self sustained sequence replication (Guatelli, JC. et al., (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. et al., (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177),

- 110 -

Q-Beta Replicase (Lizardi, P.M. *et al.* (1988) *Bio-Technology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to
5   those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

10   [0213]   In an alternative embodiment, mutations in a marker gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more
15   restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see,
20   for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

[0214]   In other embodiments, genetic mutations in a
25   marker gene or a gene encoding a marker protein of the invention can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin, M.T. *et al.* (1996)
30   *Human Mutation* 7: 244-255; Kozal, M.J. *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in marker can be identified in two dimensional arrays containing light generated DNA probes as described in Cronin, M.T. *et al.* *supra.*

- 111 -

Briefly, a first hybridization array of probes can be
used to scan through long stretches of DNA in a sample
and control to identify base changes between the
sequences by making linear arrays of sequential
5   overlapping probes. This step allows the
identification of point mutations. This step is
followed by a second hybridization array that allows
the characterization of specific mutations by using
smaller, specialized probe arrays complementary to all
10 variants or mutations detected. Each mutation array is
composed of parallel probe sets, one complementary to
the wild-type gene and the other complementary to the
mutant gene.

15 [0215] In yet another embodiment, any of a variety of
sequencing reactions known in the art can be used to
directly sequence the marker gene and detect mutations
by comparing the sequence of the sample marker with the
corresponding wild-type (control) sequence. Examples
20 of sequencing reactions include those based on
techniques developed by Maxam and Gilbert ((1977) *Proc.
Natl. Acad. Scl. USA* 74:560) or Sanger ((1977) *Proc.
Natl. Acad. Sci. USA* 74:5463). It is also contemplated
that any of a variety of automated sequencing
25 procedures can be utilized when performing the
diagnostic assays ((1995) *Biotechniques* 19:448),
including sequencing by mass spectrometry (see, *e.g.,*
PCT International Publication No. WO 94116101; Cohen *et
al.* (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et
30 al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

[0216] Other methods for detecting mutations in the
marker gene or gene encoding a marker protein of the
invention include methods in which protection from

- 112 -

cleavage agents is used to detect mismatched bases in
RNA/RNA or RNA/DNA heteroduplexes (Myers et al. (1985)
Science 230:1242). In general, the art technique of
"mismatch cleavage" starts by providing heteroduplexes

5   by hybridizing (labeled) RNA or DNA containing the
wild-type marker sequence with potentially mutant RNA
or DNA obtained from a tissue sample. The double-
stranded duplexes are treated with an agent which
cleaves single-stranded regions of the duplex such as

10   which will exist due to basepair mismatches between the
control and sample strands. For instance, RNA/DNA
duplexes can be treated with RNase and DNA/DNA hybrids
treated with S1 nuclease to enzymatically digest the
mismatched regions. In other embodiments, either

15   DNA/DNA or RNA/DNA duplexes can be treated with
hydroxylamine or osmium tetroxide and with piperidine
in order to digest mismatched regions. After digestion
of the mismatched regions, the resulting material is
then separated by size on denaturing polyacrylamide

20   gels to determine the site of mutation. See, for
example, Cotton et al. (1988) Proc. Natl Acad Sci USA
85:4397; Saleeba et al. (1992) Methods Enzymol.
517:286-295. In a preferred embodiment, the control
DNA or RNA can be labeled for detection.

25

[0217]   In still another embodiment, the mismatch
cleavage reaction employs one or more proteins that
recognize mismatched base pairs in double-stranded DNA
(so called "DNA mismatch repair" enzymes) in defined

30   systems for detecting and mapping point mutations in
marker cDNAs obtained from samples of cells. For
example, the mutY enzyme of E. coli cleaves A at G/A
mismatches and the thymidine DNA glycosylase from HeLa
cells cleaves T at G/T mismatches (Hsu et al. (1994)

- 113 -

Carcinogenesis 15:1657-1652). According to an
exemplary embodiment, a probe based on a marker
sequence, *e.g.*, a wild-type marker sequence, is
hybridized to a cDNA or other DNA product from a test
5 cell(s). The duplex is treated with a DNA mismatch
repair enzyme, and the cleavage products, if any, can
be detected from electrophoresis protocols or the like.
See, for example, U.S. Patent No. 5,459,039.

10 [0218] In other embodiments, alterations in
electrophoretic mobility will be used to identify
mutations in marker genes or genes encoding a marker
protein of the invention. For example, single strand
conformation polymorphism (SSCP) may be used to detect
15 differences in electrophoretic mobility between mutant
and wild type nucleic acids (Orita *et al.* (1989) *Proc
Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993)
Mutat. Res. 285:125-144; and Hayashi (1992) *Genet.
Anal. Tech Appl.* 9:73-79). Single-stranded DNA
20 fragments of sample and control marker nucleic acids
will be denatured and allowed to renature. The
secondary structure of single-stranded nucleic acids
varies according to sequence, the resulting alteration
in electrophoretic mobility enables the detection of
25 even a single base change. The DNA fragments may be
labeled or detected with labeled probes. The
sensitivity of the assay may be enhanced by using RNA
(rather than DNA), in which the secondary structure is
more sensitive to a change in sequence. In a preferred
30 embodiment, the subject method utilizes heteroduplex
analysis to separate double stranded heteroduplex
molecules on the basis of changes in elecrtophoretic
mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

- 114 -

[0219]   In yet another embodiment the movement of
mutant or wild-type fragments in polyacrylamide gels
containing a gradient of denaturant is assayed using
denaturing gradient gel electrophoresis (DGGE) (Myers
5 et al. (1985) *Nature* 313:495). When DGGE is used as
the method of analysis, DNA will be modified to insure
that it does not completely denature, for example by
adding a GC clamp of approximately 40 by of high-
melting GC-rich DNA by PCR. In a further embodiment, a
10 temperature gradient is used in place of a denaturing
gradient to identify differences in the mobility of
control and sample DNA (Rosenbaum and Reissner (1987)
Biophys Chem 265:12753).

15 [0220]   Examples of other techniques for detecting
point mutations include, but are not limited to,
selective oligonucleotide hybridization, selective
amplification, or selective primer extension. For
example, oligonucleotide primers may be prepared in
20 which the known mutation is placed centrally and then
hybridized to target DNA under conditions which permit
hybridization only if a perfect match is found (Saiki
et al. (1986) *Nature* 324:163); Saiki et al. (1989)
Proc. Natl. Acad. Sci USA 86:6230). Such allele
25 specific oligonucleotides are hybridized to PCR
amplified target DNA or a number of different mutations
when the oligonucleotides are attached to the
hybridizing membrane and hybridized with labeled target
DNA.
30

[0221]   Alternatively, allele specific amplification
technology which depends on selective PCR amplification
may be used in conjunction with the instant invention.
Oligonucleotides used as primers for specific

- 115 -

amplification may carry the mutation of interest in the
center of the molecule (so that amplification depends
on differential hybridization) (Gibbs *et al.* (1989)
Nucleic Acids Res. 17:2437-2448) or at the extreme 3'

5   end of one primer where, under appropriate conditions,
mismatch can prevent, or reduce polymerase extension
(Prossner (1993) *Tibtech* 11:238). In addition it may
be desirable to introduce a novel restriction site in
the region of the mutation to create cleavage-based

10   detection (Gasparini *et al.* (1992) *Mol. Cell Probes*
6:1). It is anticipated that in certain embodiments
amplification may also be performed using Taq ligase
for amplification (Barany (1991) *Proc. Natl. Acad. Sci
USA* 88:189). In such cases, ligation will occur only

15   if there is a perfect match at the 3' end of the 5'
sequence making it possible to detect the presence of a
known mutation at a specific site by looking for the
presence or absence of amplification.

20   [0222] The methods described herein may be performed,
for example, by utilizing prepackaged diagnostic kits
comprising at least one probe nucleic acid or antibody
reagent described herein, which may be conveniently
used, *e.g.*, in clinical settings to diagnose subjects

25   exhibiting symptoms or family history of a disease or
illness involving a marker gene.

[0223] Furthermore, any cell type or tissue in which
marker is expressed may be utilized in the prognostic
30   assays described herein.

- 116 -

3.   Monitoring of Effects During Clinical Trials

[0224]   Monitoring the influence of agents (*e.g.*,
drugs) on the expression or activity of a marker
5   protein (*e.g.*, the modulation of multiple sclerosis)
can be applied not only in basic drug screening, but
also in clinical trials. For example, the
effectiveness of an agent determined by a screening
assay as described herein to increase marker gene
10 expression, protein levels, or upregulate marker
activity, can be monitored in clinical trials of
subjects exhibiting decreased marker gene expression,
protein levels, or downruegulated marker activity.
Alternatively, the effectiveness of an agent determined
15 by a screening assay to decrease marker gene
expression, protein levels, or downregulate marker
activity, can be monitored in clinical trials of
subjects exhibiting increased marker gene expression,
protein levels, or upregulated marker activity. In
20 such clinical trials, the expression or activity of a
marker gene, and preferably, other genes that have been
implicated in, for example, a marker-associated
disorder (*e.g.*, multiple sclerosis) can be used as a
"read out" or markers of the phenotype of a particular
25 cell.

[0225]   For example, and not by way of limitation,
genes, including marker genes and genes encoding a
marker protein of the invention, that are modulated in
30 cells by treatment with an agent (*e.g.*, compound, drug
or small molecule) which modulates marker activity
(*e.g.*, identified in a screening assay as described
herein) can be identified. Thus, to study the effect
of agents on marker-associated disorders (*e.g.*,

- 117 -

multiple sclerosis), for example, in a clinical trial,
cells can be isolated and RNA prepared and analyzed for
the levels of expression of marker and other genes
implicated in the marker-associated disorder,

5   respectively. The levels of gene expression (e.g., a
gene expression pattern) can be quantified by northern
blot analysis or RT-PCR, as described herein, or
alternatively by measuring the amount of protein
produced, by one of the methods as described herein, or

10 by measuring the levels of activity of marker or other
genes. In this way, the gene expression pattern can
serve as a marker, indicative of the physiological
response of the cells to the agent. Accordingly, this
response state may be determined before, and at various

15 points during treatment of the individual with the
agent.

[0226] In a preferred embodiment, the present
·invention provides a method for monitoring the

20 effectiveness of treatment of a subject with an agent
(e.g., an agonist, antagonist, peptidomimetic, protein,
peptide, nucleic acid, small molecule, or other drug
candidate identified by the screening assays described
herein) including the steps of (i) obtaining a pre-

25 administration sample from a subject prior to
administration of the agent; (ii) detecting the level
of expression of a marker protein, mRNA, or genomic DNA
in the pre-administration sample; (iii) obtaining one
or more post-administration samples from the subject;

30 (iv) detecting the level of expression or activity of
the marker protein, mRNA, or genomic DNA in the post-
administration samples; (v) comparing the level of
expression or activity of the marker protein, mRNA, or
genomic DNA in the pre-administration sample with the

35 marker protein, mRNA, or genomic DNA in the post

- 118 -

administration sample or samples; and (vi) altering the
administration of the agent to the subject accordingly.
For example, increased administration of the agent may
be desirable to increase the expression or activity of

5   marker to higher levels than detected, *i.e.*, to
increase the effectiveness of the agent.
Alternatively, decreased administration of the agent
may be desirable to decrease expression or activity of
marker to lower levels than detected, *i.e.* to decrease

10   the effectiveness of the agent. According to such an
embodiment, marker expression or activity may be used
as an indicator of the effectiveness of an agent, even
in the absence of an observable phenotypic response.

15   C.   Methods of Treatment

     [0227] The present invention provides for both
     prophylactic and therapeutic methods of treating a
     subject at risk for (or susceptible to) a disorder or

20   having a disorder associated with aberrant marker
     expression or activity. With regards to both
     prophylactic and therapeutic methods of treatment, such
     treatments may be specifically tailored or modified,
     based on knowledge obtained from the field of

25   pharmacogenomics. "Pharmacogenomics", as used herein,
     includes the application of genomics technologies such
     as gene sequencing, statistical genetics, and gene
     expression analysis to drugs in clinical development
     and on the market. More specifically, the term refers

30   the study of how a subject's genes determine his or her
     response to a drug (*e.g.*, a subject's "drug response
     phenotype", or "drug response genotype".) Thus,
     another aspect of the invention provides methods for
     tailoring an individual's prophylactic or therapeutic

35   treatment with either the marker molecules of the

- 119 -

present invention or marker modulators according to
that individual's drug response genotype.
Pharmacogenomics allows a clinician or physician to
target prophylactic or therapeutic treatments to
5   subjects who will most benefit from the treatment and
to avoid treatment of subjects who will experience
toxic drug-related side effects.

        1.   Prophylactic Methods
10

    [0228]   In one aspect, the invention provides a method
    for preventing in a subject, a disease or condition
    (e.g., multiple sclerosis) associated with increased or
    decreased marker expression or activity, by
15   administering to the subject a marker protein or an
    agent which modulates marker protein expression or at
    least one marker protein activity. Subjects at risk
    for a disease which is caused or contributed to by
    increased or decreased marker expression or activity
20   can be identified by, for example, any or a combination
    of diagnostic or prognostic assays as described herein.
    Administration of a prophylactic agent can occur prior
    to the manifestation of symptoms characteristic of the
    differential marker protein expression, such that a
25   disease or disorder is prevented or, alternatively,
    delayed in its progression. Depending on the type of
    marker aberrancy (e.g., increase or decrease in
    expression level), for example, a marker protein,
    marker protein agonist or marker protein antagonist
30   agent can be used for treating the subject. The
    appropriate agent can be determined based on screening
    assays described herein.

- 120 -

2.   Therapeutic Methods

[0229] Another aspect of the invention pertains to
methods of modulating marker protein expression or
5   activity for therapeutic purposes. Accordingly, in an
exemplary embodiment, the modulatory method of the
invention involves contacting a cell with a marker
protein or agent that modulates one or more of the
activities of a marker protein activity associated with
10   the cell. An agent that modulates marker protein
activity can be an agent as described herein, such as a
nucleic acid or a protein, a naturally-occurring target
molecule of a marker protein (e.g., a marker protein
substrate), a marker protein antibody, a marker protein
15   agonist or antagonist, a peptidomimetic of a marker
protein agonist or antagonist, or other small molecule.
In one embodiment, the agent stimulates one or more
marker protein activities. Examples of such
stimulatory agents include active marker protein and a
20   nucleic acid molecule encoding marker protein that has
been introduced into the cell. In another embodiment,
the agent inhibits one or more marker protein
activities. Examples of such inhibitory agents include
antisense marker protein nucleic said molecules, anti-
25   marker protein antibodies, and marker protein
inhibitors. These modulatory methods can be performed
in vitro (e.g., by culturing the cell with the agent)
or, alternatively, in vivo (e.g., by administering the
agent to a subject). As such, the present invention
30   provides methods of treating an individual afflicted
with a disease or disorder characterized by aberrant
expression or activity of a marker protein or nucleic
acid molecule. In one embodiment, the method involves
administering an agent (e.g., an agent identified by a
35   screening assay described herein), or combination of

- 121 -

agents that modulates (*e.g.*, upregulates or
downregulates) marker protein expression or activity.
In another embodiment, the method involves
administering a marker protein or nucleic acid molecule
5   as therapy to compensate for reduced or aberrant marker
protein expression or activity.

[0230]   Stimulation of marker protein activity is
desirable in situations in which marker protein is
10   abnormally downregulated and/or in which increased
marker protein activity is likely to have a beneficial
effect. For example, stimulation of marker protein
activity is desirable in situations in which a marker
is downregulated and/or in which increased marker
15   protein activity is likely to have a beneficial erect.
Likewise, inhibition of marker protein activity is
desirable in situations in which marker protein is
abnormally upregulated and/or in which decreased marker
protein activity is likely to have a beneficial effect.
20

     3.   Pharmacogenomics

[0231]   The marker protein and nucleic acid molecules
of the present invention, as well as agents, inhibitors
25   or modulators which have a stimulatory or inhibitory
effect on marker protein activity (*e.g.*, marker gene
expression) as identified by a screening assay
described herein can be administered to individuals to
treat (prophylactically or therapeutically) marker-
30   associated disorders (*e.g.*, multiple sclerosis)
associated with aberrant marker protein activity. In
conjunction with such treatment, pharmacogenomics
(*i.e.*, the study of the relationship between an
individual's genotype and that individual's response to

- 122 -

a foreign compound or drug) may be considered.
Differences in metabolism of therapeutics can lead to
severe toxicity or therapeutic failure by altering the
relation between dose and blood concentration of the
5   pharmacologically active drug. Thus, a physician or
clinician may consider applying knowledge obtained in
relevant pharmacogenomics studies in determining
whether to administer a marker molecule or marker
modulator as well as tailoring the dosage and/or
10 therapeutic regimen of treatment with a marker molecule
or marker modulator.

[0232] Pharmacogenomics deals with clinically
significant hereditary variations in the response to
15 drugs due to altered drug disposition and abnormal
action in affected persons. See, for example,
Eichelbaum, M. *et al.* (1996) *Clin. Exp. Pharmacol.
Physiol.* 23(10-11) :983-985 and Linden, M.W. *et al.*
(1997) *Clin. Chem.* 43(2):254-266. In general, two
20 types of pharmacogenetic conditions can be
differentiated. Genetic conditions transmitted as a
single factor altering the way drugs act on the body
(altered drug action) or genetic conditions transmitted
as single factors altering the way the body acts on
25 drugs (altered drug metabolism). These pharmacogenetic
conditions can occur either as rare genetic defects or
as naturally-occurring polymorphisms. For example,
glucose-6-phosphate dehydrogenase deficiency (G6PD) is
a common inherited enzymopathy in which the main
30 clinical complication is haemolysis after ingestion of
oxidant drugs (anti-malarials, sulfonamides,
analgesics, nitrofurans) and consumption of fava beans.

[0233] One pharmacogenomics approach to identifying
35 genes that predict drug response, known as "a genome-

- 123 -

wide association", relies primarily on a high-
resolution map of the human genome consisting of
already known gene-related markers (e.g., a "bi-
allelic" gene marker map which consists of 60,000-
5   100,000 polymorphic or variable sites on the human
genome, each of which has two variants.) Such a high-
resolution genetic map can be compared to a map of the
genome of each of a statistically substantial number of
subjects taking part in a Phase II/III drug trial to
10   identify markers associated with a particular observed
drug response or side effect. Alternatively, such a
high resolution map can be generated from a combination
of some ten-million known single nucleotide
polymorphisms (SNPs) in the human genome. As used
15   herein, a "SNP" is a common alteration that occurs in a
single nucleotide base in a stretch of DNA. For
example, a SNP may occur once per every 1000 bases of
DNA. A SNP may be involved in a disease process,
however, the vast majority may not be disease
20   associated. Given a genetic map based on the
occurrence of such SNPs, individuals can be grouped
into genetic categories depending on a particular
pattern of SNPs in their individual genome. In such a
manner, treatment regimens can be tailored to groups of
25   genetically similar individuals, taking into account
traits that may be common among such genetically
similar individuals.

[0234] Alternatively, a method termed the "candidate
30   gene approach", can be utilized to identify genes that
predict drug response. According to this method, if a
gene that encodes a drugs target is known (e.g., a
marker protein of the present invention), all common
variants of that gene can be fairly easily identified
35   in the population and it can be determined if having

- 124 -

one version of the gene versus another is associated
with a particular drug response.

[0235]   As an illustrative embodiment, the activity of
drug metabolizing enzymes is a major determinant of
both the intensity and duration of drug action. The
discovery of genetic polymorphisms of drug metabolizing
enzymes (e.g., N-acetyltransferase 2 (NAT 2) and
cytochrome P450 enzymes CYP2D6 and CYPZC19) has
provided an explanation as to why some subjects do not
obtain the expected drug effects or show exaggerated
drug response and serious toxicity after taking the
standard and safe dose of a drug. These polymorphisms
are expressed in two phenotypes in the population, the
extensive metabolizer (EM) and poor metabolizer (PM).
The prevalence of PM is different among different
populations. For example, the gene coding for CYP2D6
is highly polymorphic and several mutations have been
identified in PM, which all lead to the absence of
functional CYP2D6. Poor metabolizers of CYP2D6 and
CYP2C19 quite frequently experience exaggerated drug
response and side effects when they receive standard
doses. If a metabolite is the active therapeutic
moiety, PM show no therapeutic response, as
demonstrated for the analgesic effect of codeine
mediated by its CYP2D6-formed metabolite morphine. The
other extreme are the so called ultra-rapid
metabolizers who do not respond to standard doses.
Recently, the molecular basis of ultra-rapid metabolism
has been identified to be due to CYP2D6 gene
amplification.

[0236]   Alternatively, a method termed the "gene
expression profiling", can be utilized to identify
genes that predict drug response. For example, the

- 125 -

gene expression of an animal dosed with a drug (e.g., a marker molecule or marker modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

5

[0237] Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment an individual.
10 This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a marker molecule or marker modulator, such as a modulator
15 identified by one of the exemplary screening assays described herein.

[0238] This invention is further illustrated by the following examples which should not be construed as
20 limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Figures and Tables are incorporated herein by reference.

- 126 -

EXAMPLES

EXAMPLE 1: IDENTIFICATION AND CHARACTERIZATION OF
MARKER cDNA IN MURINE MODEL OF EXPERIMENTAL AUTOIMMUNE
5   ENCHEPHALITIS (EAE)

A.   Induction of EAE in Mice

[0239]   12 SJL mice were immunized subcutaneously with
10 150 μg injection of proteolipid protein (PLP), peptide
residues 139-151 in complete Freud's adjuvant, a
protein capable of inducing EAE in mice. After 10
days, the spleens were harvested, and splenocyte cells
were isolated and reactivated *in vitro* for 4 days with
15 5 μg of PLP. The amplified splenocyte cells were then
injected into SJL mice at 15 x 10^6 cells per mouse.

B.   *In Vivo* Development of EAE and Isolation of Immune
Cells and Microglial Cells
20

[0240]   Development of EAE in mice was characterized by
three stages: onset, peak and recovery. Onset of EAE
in mice was characterized by a limp tail 7-8 days after
injection. Peak development of EAE in mice appeared as
25 full hind limb paralysis approximately 10-12 days after
injection, while recovery appeared as hind limb
weakness approximately 16-18 days after injection. At
each of the above 3 stages, the following numbers of
mice were sacrificed and harvested for cervical spinal
30 cord and mid-brain: 2 mice at onset, 6 at peak and 4
at recovery. To isolate infiltrating immune cells and
microglial cells, the samples were run over a percoll
gradient to remove neurons, endothelial cells and
astrocytes.
35

- 127 -

C. Isolation of RNA

[0241] Total RNA was isolated using the RNeasy mini
kit (Quiagen, Hilden, Germany). To prepare cRNA for
5   hybridization, 5 μg of total RNA was denatured at 70°C
with T7-tagged oligo-dT primer, cooled on ice, then
reverse transcribed with 200 units Superscript RT II at
50°C for 1 hour in 1x first strand buffer, 10 mM DTT
and 0.5 mM of each dNTP (Gibco BRL, Gaiethersburg, MD).
10 Second strand cDNA was synthesized by adding 40 units
DNA pol I, 10 units *E. coli* DNA ligase, 2 units Rnase
H, 30 μL second strand buffer, 3 μl 10 mM each dNTP,
and water to 150 μL final volume and incubating at
15.8°C for 2 hours. The resulting cDNA was extracted
15 once with phenol/chloroform/isoamylalcohol. CDNA was
separated on a Phase Lock Gel tube at maximum speed for
2 min and precipitated with sodium acetate and 100
ethanol. The resulting pellet was washed with 80%
ethanol, was dried and was resuspended in
20 diethylpyrocarbonate-treated (DEPC-treated) water.

[0242] Labeled RNA was prepared from clones containing
a T7 RNA polymerase promoter site by incorporating
labeled ribonucleotides in an *in vitro* transcription
25 (IVT) reaction. Half of the purified cDNA was used for
in vitro transcription with a T7 RNA polymerase kit,
following manufacturer instructions and using an
overnight 37 °C incubation, thereby incorporating
biotinylated CTP and UTP. Labeled RNA was purified
30 using RNeasy columns (Quiagen). RNA was concentrated
and then quantitated by spectrophotometry. Labeled RNA
(13-15 μg) was fragmented in 40 mM Tris-acetate 8.0,
100 mM potassium acetate, 30 mM magnesium acetate for
35 min at 94 C in a total volume of 40 μL.

35

- 128 -

D. Array Hybridization and Detection of Fluorescence

[0243] The labeled and fragmented RNA probes were
diluted in 1 x MES buffer, BIO948, Bio C, B cre, 100
5   μg/ml herring sperm DNA, and 50 μg/ml acetylated BSA.
New probes were pre-hybridized in a microfuge tube with
glass beads at 45°C overnight to remove debris.
Oligonucleotide arrays composed of approximately 11,000
murine genes (Microarray, Affymetrix, Cat Nos. SubA
10 #510243, SubB #510244) were pre-hybridized with 1 x MES
hybridization buffer at 45°C for 5 min and then
insoluble material was removed by centrifugation. Pre-
hybridization buffer was removed from oligo array
cartridges, 200 μL probe added and cartridges were
15 hybridized for 16 hours at 45°C at 60 rpm. After
hybridization, probes were removed and the cartridges
washed extensively with 6 x SSPET using a fluidics
station (Affymetrix). Following hybridization, the
solutions were removed, the arrays were washed with 6x
20 SSPE-T at 22°C for 7 min, and then washed with 0.5 x
SSPE-T at 40°C for 15 minutes. When biotin-labeled RNA
was used, the hybridized RNA was stained with a
streptavidin-phycoerythrin conjugate (Molecule Probes,
Eugene, OR) prior to reading. Hybridized arrays were
25 stained with 2 μg/ml streptavidin-phycoerythrin in 6x
SSPE-T at 40°C for 5 minutes. The arrays were read
using a scanning confocal microscope made for
Affymetrix by Molecular Dynamics (commercially
available through Affymetrix, Santa Clara, CA). The
30 scanner uses an argon ion laser as the excitation
source, with the emission detected by a photomultiplier
tube through either a 530 nm bandpass filter
(fluorescein), or a 560 nm longpass filter
(phycoerythrin). Nucleic acids of either sense or
35 antisense orientations were used in hybridization

experiments. Arrays with probes for either orientation
(reverse complements of each other) are made using the
same set of photolithographic masks by reversing the
order of the photochemical steps and incorporating the
5 complementary nucleotide.

E. Quantitative Analysis of Hybridization Patterns and
Insensitivities

10 [0244] Following a quantitative scan of an array, or
biochip, a grid is aligned to the image using the known
dimensions of the array and the corner control regions
as markers. The image is reduced to a simple text file
containing position and intensity information using
15 software developed at Affymetrix (GENECHIP 3.0
software). This information is merged with another
text file that contains information relating physical
position on the array to probe sequence and the
identity of the RNA and the specific part of the RNA
20 for which the oligonucleotide probe is designed. The
quantitative analysis of the hybridization results
involves a simple form of pattern recognition based on
the assumption that, in the presence of a specific RNA,
the PM probes will hybridize more strongly on average
25 than their MM partners. The number of instances in
which the PM hybridization signal is larger than the MM
signal is computed along with the average of the
logarithm of the PM/MM ratios for each probe set.
These values are used to make a decision (using a
30 predefined decision matrix) concerning the presence or
absence of an RNA. To determine the quantitative RNA
abundance, the average of the differences (PM minus MM)
for each probe family is calculated. The advantage of
the difference method is that signals from random
35 cross-hybridization contribute equally, on average, to

- 130 -

the PM and MM probes, while specific hybridization
contributes more to the PM probes. By averaging the
pairwise differences, the real signals add
constructively while the contributions from cross-
5 hybridization tend to cancel. When assessing the
differences between two different RNA samples, the
hybridization signals from side-by-side experiments on
identically synthesized arrays are compared directly.
The magnitude of the changes in the average of the
10 difference (PM-MM) values is interpreted by comparison
with the results of spiking experiments as well as the
signals observed for the internal standard bacterial
and phage RNAs spiked into each sample at a known
amount. Data analysis programs developed at
15 Affymetrix, such as the GENECHIP 3.0 software, perform
these operation automatically.

[0245] Over 5000 genes were expressed in the central
nervous system of the murine samples. Nonetheless,
20 distinct gene expression patterns emerged between peak
disease and onset or recovery of the disease. In order
to identify the most active genes in EAE development,
genes were sought which revealed a pattern of increased
or decreased regulation at peak, as compared to onset
25 or recovery. The onset stage served as the baseline
comparison, with the assumption that the onset stage
was similar in gene expression to undifferentiated
naive T cells (i.e., similar to normal control samples
samples). The genes demonstrating at least a two-fold
30 increase at peak in EAE tissue as opposed to non-
involved tissue are set forth in Table 1, whereas genes
having a decrease in expression in EAE tissue as
opposed to non-involved tissue are set forth in Table
2. There are several genes which were previously known
35 to be associated with EAE (e.g., which are differently

- 131 -

expressed in MS tissue): TNF-α and IFN-β. The up-
regulation of these genes served as validation to the
method.

5   [0246] To generate the data in Tables 1 and 2, genes
were clustered hierarchically into groups on the basis
of similarity of their expression profiles by the
procedure of Eisen et al. ((1998) Proc. Nat'l Acad.
Sci. USA 95:25: 14863-8). Genes that were designated
10 absent (A) in all samples in a given experiment were
eliminated from the analysis, as were -fold changes
over the designated baseline of less than 2. Genes
that were present (P) and were of unclear expression
(M) were also indicated. Average fold changes between
15 peak/onset and peak/recovery stages were also
calculated.

F. Identification of Immune Mediated Genes by
20 Comparison to *In Vitro* Expression Patterns

[0247] In addition, to identify disease-related,
antigen-driven immune mediated genes, additional mice
were immunized with an EAE-inducing factor and tissue
25 harvested for splenocytes. 3 SJL mice were immunized
on Day 0 with 400 μg pertussis toxin and 200 μg
injection of myelin oligodendrocyte glycoprotein (MOG)
peptide residue 35-55 which, like PLP, induces EAE in
mice. On Day 3, an additional dose of 400 μg pertussis
30 toxin was administered. After 10 days, the spleens
were harvested, and splenocyte cells were isolated and
cultured *in vitro* with 5 μg/ml MOG for 6 and 24 hours,
to monitor expression of an immune response at an
earlier time (6 hours) and at a later time (24 hours).
35 In addition, a subset of the splenocyte cells were

- 132 -

further treated with 5 μg/ml of anti-IL12p40 (C17.8)
antibody, which is believed to produce inhibitory
effects on the expression of MS (IL12p40 is an
interleukin that induces IFN-γ expression in the Th-1
5 pathway).

[0248] Isolation of mRNA was performed as described
above, with the *in vitro* samples being compared to
murine 11,000 genes (Microarray, Affymetrix, Cat Nos.
10 SubA #510243, SubB #510244). As shown in Table 3, MOG
restimulation induced differential expression of 199
genes. Of these genes, Tables 4 and 5 indicate 17
genes which further responded to treatment with anti-
IL12p40. In addition, quantitative analysis was
15 performed by comparison of the *in vitro* samples to the
in vivo studies, to identify common genes which were
increased or decreased substantially, and/or which were
affected by addition by MOG + antiIL12p40. Of the 1265
genes which were found to increase or decrease greater
20 than three fold following MOG restimulation, 184 were
found to be in common with genes expressed at peak
disease of EAE. Further analysis revealed 6 genes in
particular which also responded to treatment with anti-
IL12p40 (shown in Table 5): CAPN12, MT1, MYO1F, TLN,
25 UNK_AA117532 and UNK_AA645990.

EXAMPLE 2: IDENTIFICATION AND CHARACTERIZATION OF
MARKER cDNA IN HUMAN MODEL OF MULTIPLE SCLEROSIS.

30 A. Study Design and Subject Entry Criteria

[0249]   60 patients diagnosed with varying stages of
multiple sclerosis participated in the study from three
sites: University Rochester (Rochester, NY), Lehigh
35 Valley Hospital (Allentown, PA) and Institute of

- 133 -

Neurology, University of London (London, UK). The
University of Rochester and Lehigh Valley Hospital
provided peripheral blood mononuclear cell (PBMC)
samples, while the Institute of Neurology provided
5 brain lesion and non-lesional samples.

[0250] As is characteristic of MS, the patients
manifested other neurological disorders and a wide
panoply of symptoms, and were at different stages of
10 the disease: relapsing-remitting, 2° progressive, 1°
progressive and acute exacerbation. The samples
included post-mortem lesional and nonlesional brain
samples, as well as other normal controls.

15 [0251] In general, the donor profile for PBMC samples
had the following requirements: no current use of
steroids (washout of 30 days), no current use of
interferon or copaxone (washout 90 days), no previous
history of cyclophosphamide use, no active viral
20 illnesses or infections and no current use of any other
study drug.

B.   Identification of MS markers from Blood or Brain

25 [0252] PBMC were isolated from blood samples using
Lymphoprep (Nycomed, Oslo, Norway). Lesion and
nonlesion brain samples were removed from brain stems
and washed   To isolate infiltrating immune cells and
microglial cells, the samples were run over percoll
30 gradient to remove neurons, endothelial cells and
astrocytes.

[0253] As was performed in Experiment 1(C) above, RNA
was isolated from the PBMC and brain samples using the

- 134 -

RNeasy mini kit (Quiagen), the RNA probes were
hybridized against human chip arrays having
approximately 14,000 genes (MicroArray, Affymetrix, cat
no. 510448) and quantitative analysis was performed
5   using GeneChip. PBMC samples from MS patients were
compared to PBMCs from undiseased pateints, resulting
in over 300 differentially regulated genes by more than
2 fold (p<.01), as shown in Table 7. Lesions from the
MS brain were compared to nonlesion samples, resulting
10   in over 100 genes, as shown in Table 8, that increased
or decreased more than 2 fold (p<.01). A comparison of
the genes expressed in brain and PBMC samples indicated
that only 181 genes were shown to be present in both.

15   C.   Comparison to *In Vitro* Expression Patterns

[0254]   These genes were compared to genes identified
in the murine samples of Example 1(F). Of the 181
genes which were either up- or down-regulated in the
20   human PBMC and brain samples, 6 genes were shown to be
in common with the murine genes that were
differentially regulated *in vitro*: EEF1D, PIM2, PRDX2,
SEC24C, UNK_AJ24 AND XIP (these genes and their
accession numbers are listed in Table 9).

25

D.   Taqman Polymerase Chain Reaction

[0255]   To ensure that the data obtained from the
GeneChip analysis (described above) was reflective of
30   the actual level of gene expression in the cell
samples, the expression of 4 selected cytokines, IL-10,
IL-8, IL-12p35 and IL-1β, were also measured by
polymerase chain reaction, and the results compared to
the expression levels of the same cDNAs of the
35   cytokines in non-involved samples and in GeneChip

Table 3

UNK_AA013783	Msa.5720.0	8.5	45	22	0.49	ESTs, Highly similar to INTERFERON-INDUCED 35 KD PROTEIN [Homo sapiens]
DAXX	AF006040	26	68	33	0.49	Fas death domain-associated protein
CASP8	Msa.28381.0	5	17	8	0.47	caspase 8
FAS	FAS	6	17	8	0.47	FAS control sequence (M. musculus) [AFFX]
UNK_ET61797	ET61797	6	13	6	0.46	Mus musculus anti-DNA immunoglobulin heavy chain IgG mRNA, antibody 363s.62, partial cds.
UNK_X52622	Msa.2275.0	4	9	4	0.44	Mouse IN gene for the integrase of an endogenous retrovirus
UNK_ET62928	ET62928	9	16	7	0.44	M.musculus antibody heavy chain variable region (366bp).
HDC	X57437	14.5	55	24	0.44	histidine decarboxylase cluster
ISG15	x56602	83	381	163	0.43	interferon-stimulated protein (15 kDa)
UNK_AA163967	Msa.41726.0	4.5	12	5	0.42	mr24g07.r1 Mus musculus cDNA, 5' end
UNK_AA072573	Msa.28697.0	5.5	46	19	0.41	pre-B-cell colony-enhancing factor
BACTIN5_ST_Mm_	AFFX-b-ActinMurM1	7.5	17	7	0.41	Beta-actin 5' control sequence (M. musculus) [AFFX]
TNF	x02811	8	42	17	0.40	tumor necrosis factor
CDKN1A	Msa.1382.0	7.5	23	9	0.39	cyclin-dependent kinase inhibitor 1A (P21)
UBL1A2-PENDING	Msa.20430.0	10.5	26	10	0.38	ubiquitin-like 1 (sentrin) activating enzyme subunit 2
IFIT2	U43085	15.5	94	36	0.38	Interferon-induced protein with tetratricopeptide repeats 2
UNK_L32973	L32973	33	130	48	0.37	thymidylate kinase homologue
USP18	aa286391	21.5	106	39	0.37	ubiquitin specific protease 18
BACTIN5_ST_Mm_	AFFX-b-ActinMurM1	6.5	14	5	0.36	Beta-actin 5' control sequence (M. musculus) [AFFX]
ACTX	Msa.32904.0	47	174	62	0.36	melanoma X-actin
SCYA4	m35590	4.5	17	6	0.35	small inducible cytokine A4
L-CCR	Msa.22422.0	13	111	37	0.33	lipopolysaccharide inducible C-C chemokine receptor related
RGS1	Msa.37568.0	5	12	4	0.33	regulator of G-protein signaling 1
MAPK13	Msa.6386.0	5	12	4	0.33	mitogen activated protein kinase 13
SCYB10	m33266	14	265	78	0.29	small inducible cytokine B subfamily (Cys-X-Cys), member 10
TNF	TNFa	5	24	7	0.29	TNF control sequence (M. musculus) [AFFX]
IFIT3	l32974	43.5	182	53	0.29	interferon-induced protein with tetratricopeptide repeats 3
MX1	m12279	7	36	10	0.28	myxovirus (influenza virus) resistance 1
ICAM1	x52264	5.5	44	12	0.27	intercellular adhesion molecule
TNF	TNFa	4.5	30	8	0.27	TNF control sequence (M. musculus) [AFFX]
UNK_ET61450	ET61450	4	15	4	0.27	Mus musculus interleukin-2 (IL-2) mRNA, strain-specific RF allele, partial cds.
IFIT1	U43084	27	131	32	0.24	interferon-induced protein with tetratricopeptide repeats 1
UNK_X73040	IL2	4	19	4	0.21	IL2 control sequence (M. musculus) [AFFX]
L-CCR	AF030185	5	24	5	0.21	lipopolysaccharide inducible C-C chemokine receptor related
UNK_ET63426	ET63426	5	29	6	0.21	Mouse strain NOD tumor necrosis factor alpha (TNFA) gene, exon 2 and 3.
IFIT1	aa616578	35.5	179	37	0.21	interferon-induced protein with tetratricopeptide repeats 1
UNK_X73040	IL2	4	23	9	0.17	IL2 control sequence (M. musculus) [AFFX]
MAD	x83106	9	78	9	0.12	Max dimerization protein
MAPKAPK2	Msa.2435.0	8.5	51	5	0.10	MAP kinase-activated protein kinase 2
UNK_D19407	D19407	4	42	4	0.10	MUSGS00778 Mouse 3'-directed cDNA; MUSGS00778; clone mb0657.

Table 4

MOG Induced and Blocked by anti-IL12

Name	Accession No.	Baseline	MOG	WT+MOG+antiIL12p40
D2WSU58E	aa409818	9	7	5
TCFL1	Msa.6010.0	12	17	14
BPGM	X13586	75	62	82
UNK_W10325	w10325	10	7	9
MRJ-PENDING	aa499641	12	12	13
ANXA4	Msa.10010.0	4	6	5
TAF2E	D49439	5	8	4
UNK_AA408555	aa408555	9	6	4
SCD2	w41032	5	6	6
UBCE7	w78245	16	17	13
UNK_AA254104	aa254104	15	4	5
UNK_ET61871	ET61871	7	16	5
UNK_AA472320	aa472320	17	16	15
UNK_AA213318	aa213318	11	11	11
UNK_ET61737	ET61737	20	21	22
UNK_AA198324	AA198324	13	17	13
SDF2	D50646	17	10	11

Table 5

Accession No.	Name	Baseline	MOG	WT+MOG+antiIL12p40	
w64452	CAPN12	5	11	5	calpain 12
V00835	MT1	4	12	4	metallothionein 1
Msa.3234.0	MYO1F	5	16	8	myosin If
x56123-2	TLN	4	16	8	talin
aa645990	UNK_AA645990	4	19	7	ESTs, Highly similar to GLUTAMINYL-TRNA SYNTHETASE [Homo sapiens]
aa117532	UNK_AA117532	7	41	16	ESTs, Moderately similar to HYPOTHETICAL 16.7 KD PROTEIN MRP17-MET14 INTERGENIC REGION [Saccharomyces cerev

Table 6

	A	B	C	D	E
1	Table of Genes Known to be Associated with MS				
2					
3	IL-12p40				
4	IL-10				
5	ICAM-2				
6	GAP-43				
7	a-2 chimmaerin				
8	myelin-transcription factor-1				
9	activin type II receptor				
10	retinoic acid receptor α-1				
11	EF-1				
12	neuron-specific enolase				
13	proton ATPase				
14	Glutaminyl-tRNA synthetase				
15	Na, K-ATPase beta subunit				
16	neuroendocrine-specific protein A				
17	alpha-N-acetylgalactosaminidase				
18	liver type alkaline phosphatase				
19	male-enhanced antigen				
20	mitochondrial enoyl-CoA hydratase				
21	import precursor-ATP synthase α				
22	serine dehydratase				
23	adenine nucleotide translocator-2				
24	ornithine decoboxylase antienzyme				
25	protein kinase C type β-1				
26	cell surface glycoprotein A15				
27	IRF-2				
28	56-K autoantigen annexin XI				
29	high endothelial venule				
30	leukotriene A-4 hydrolase				
31	transcription factor E2A				
32	TNFα receptor-2				
33	duffy chemokine receptor				
34	MS-1				
35	PRG-2				
36	MBP				
37	protein tyrosine phosphatase, receptor type, C				
38	MOG				
39	Interferon β1				
40	ICAM-1				
41	CTLA4				
42	APOE				

Table 7

Gene Name	Accession #	MS Avg	MS SD	Norm Avg	Norm SD	p value	FC	MS/No Description
XAP4	U67322	53.20	18.63	3.77	3.03	0.0039	14.11	HBV associated factor
OA48-18	AF069250	52.60	18.97	6.46	4.82	0.0050	8.14	acid-inducible phosphoprotein
KIAA0313	AB002311	13.00	5.34	2.08	1.38	0.0095	6.26	PDZ domain containing guanine nucleotide exchange factor(GEF)11; RA(Ras/Rap1A-associating)-GEF
PPP3CC	S46622	68.60	14.36	12.00	7.72	0.0004	5.72	protein phosphatase 3 (formerly 2B), catalytic subunit, gamma isoform (calcineurin A gamma)
KIAA0742	AB018285	81.80	18.99	15.54	8.07	0.0010	5.26	protein KIAA0742 protein
UNK_AL02229;	AL022398	21.00	5.05	5.65	4.18	0.0009	3.59	Cluster Incl AL022398: Homo sapiens DNA sequence from PAC 434O14 on chromosome 1q32.3.-41. Contains the HSD11B1 gene for Hydroxysteroid (11-beta
LSFR2	R56605	19.00	5.52	5.54	5.06	0.0023	3.43	linked to Surfeit genes in Fugu rubripes 2; LSFR2 gene 2
UNK_AI870785	AI870788	19.80	5.72	5.85	4.54	0.0027	3.39	Homo sapiens cDNA FLJ13967 fis, clone Y79AA1001402, weakly similar to Homo sapiens paraneoplastic cancer-testis-brain antigen (MA4) mRNA
TBCC	U61234	16.20	4.87	4.85	3.51	0.0036	3.34	tubulin-specific chaperone c
M6A	AF014837	24.80	9.07	7.46	8.69	0.0078	3.32	putative methyltransferase
BCR	X02596	18.00	4.69	5.85	4.79	0.0015	3.08	breakpoint cluster region
RAB27A	U57094	32.40	4.83	10.54	6.85	0.0000	3.07	RAB27A, member RAS oncogene family
101F6	AF040704	13.40	4.22	4.38	3.12	0.0053	3.06	putative tumor suppressor
PAP	X76770	20.40	6.62	6.77	9.36	0.0057	3.01	poly(A) polymerase
HNRPH3	AF052131	23.80	4.97	7.92	6.37	0.0003	3.00	heterogeneous nuclear ribonucleoprotein H3 (2H9)
DMP1	AI656421	16.00	6.19	10.92	8.99	0.0001	2.98	cyclin D-binding Myb-like protein
LOC56270	AF091083	14.00	2.92	4.69	2.36	0.0006	2.98	hypothetical protein 628
ARCN1	X81198	12.40	2.88	4.23	3.06	0.0008	2.93	archain 1
OGT	U77413	16.40	3.44	5.62	3.28	0.0005	2.92	O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase)
GLS	AB020645	74.20	13.83	23.62	16.07	0.0002	2.90	glutaminase
PRKACB	M34181	17.60	4.56	6.08	5.50	0.0015	2.90	protein kinase, cAMP-dependent, catalytic, beta
PTPN22	AF001846	112.80	15.01	39.00	25.96	0.0000	2.89	protein tyrosine phosphatase, non-receptor type 22 (lymphoid)
TRAF5	AB000509	52.00	14.00	18.00	9.84	0.0031	2.89	TNF receptor-associated factor 5
DKFZP564O04	AL050390	65.00	11.25	22.62	12.93	0.0001	2.87	hypothetical protein DKFZp564O043
KIAA0247	D87434	11.00	2.83	3.83	1.99	0.0025	2.86	KIAA0247 gene product
FLT3LG	U03858	36.20	7.76	12.69	12.25	0.0004	2.85	fms-related tyrosine kinase 3 ligand
APBA3	AB021638	48.40	11.41	17.00	9.84	0.0013	2.85	amyloid beta (A4) precursor protein-binding, family A, member 3 (X11-like 2)
KIAA0603	AB011175	16.20	5.17	5.69	3.45	0.0071	2.85	KIAA0603 gene product
KIAA0855	AB020682	18.80	4.21	6.62	4.07	0.0008	2.84	golgin-67
KIAA0540	AB011112	50.00	12.31	17.62	13.91	0.0012	2.84	KIAA0540 protein
HIVEP2	AL023584	12.00	2.45	4.23	2.65	0.0004	2.84	human immunodeficiency virus type I enhancer-binding protein 2
SMC1L1	D80000	27.80	7.40	9.85	8.82	0.0020	2.82	SMC1 (structural maintenance of chromosomes 1, yeast)-like 1
SULT1A2	U34804	11.20	2.28	4.00	2.16	0.0005	2.80	sulfotransferase family, cytosolic, 1A, phenol-preferring, member 2
KIAA0542	AB011114	11.60	3.05	4.15	2.97	0.0022	2.79	KIAA0542 gene product
KIAA0907	AB020714	26.80	7.96	9.54	7.48	0.0045	2.79	KIAA0907 protein
IKBKB	AF031416	21.20	6.72	7.62	5.59	0.0064	2.78	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta
POLR2H	U37689	21.40	6.95	7.69	5.08	0.0077	2.78	polymerase (RNA) II (DNA directed) polypeptide H
MYO47	AF063605	28.80	7.19	10.33	6.45	0.0018	2.77	MYO47 protein
RNF4	AB000468	61.40	14.33	22.15	12.95	0.0012	2.77	ring finger protein 4
NMT2	AF043325	13.20	4.21	4.77	2.92	0.0074	2.77	N-myristyltransferase 2
FBXO21	AB020682	10.00	3.08	3.62	1.89	0.0068	2.77	F-box only protein 21
DDX1	X70649	20.40	5.73	7.38	6.02	0.0031	2.76	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 1
HSF1	M64673	15.00	3.08	5.46	3.71	0.0004	2.75	heat shock transcription factor 1
SIP	AF080561	12.20	4.02	4.46	2.40	0.0095	2.73	SYT interacting protein
AQP3	AB001325	13.40	2.41	4.92	3.50	0.0001	2.72	aquaporin 3
MSA2	M27394	15.00	4.47	5.54	3.99	0.0049	2.71	membrane-spanning 4-domains, subfamily A, member 2
EZH1	AB002386	20.60	4.39	7.62	5.66	0.0005	2.71	enhancer of zeste (Drosophila) homolog 1
RAB2L	AL050259	17.00	1.41	6.31	5.31	0.0000	2.70	RAB2, member RAS oncogene family-like
GRB2	M96995	11.60	1.52	4.31	1.93	0.0000	2.69	growth factor receptor-bound protein 2
PSCD1	M85189	31.20	9.73	11.62	8.33	0.0064	2.69	pleckstrin homology, Sec7 and coiled/coil domains 1(cytohesin 1)
BTN3A3	U90548	18.80	4.82	7.00	6.77	0.0019	2.69	butyrophilin, subfamily 3, member A3
MPV17	X76538	22.00	3.54	8.23	6.78	0.0038	2.67	MpV17 transgene, murine homolog, glomerulosclerosis
TGFBR2	D50683	91.60	26.10	34.31	25.95	0.0002	2.67	transforming growth factor, beta receptor II (70-80kD)
RAF1	X03484	23.20	4.32	8.69	5.79	0.0000	2.67	v-raf-1 murine leukemia viral oncogene homolog 1
KIAA0129	D50919	15.60	4.39	5.85	5.59	0.0035	2.67	KIAA0129 gene product
RBM6	AF069517	24.20	7.29	9.08	6.17	0.0056	2.67	RNA binding motif protein 6
TNRC1	U60760	16.20	3.27	6.08	4.01	0.0004	2.67	trinucleotide repeat containing 1
C3ORF4	AL080097	55.20	8.58	20.77	11.60	0.0002	2.66	chromosome 3 open reading frame 4
ADH5	M81118	11.00	3.24	4.15	2.30	0.0057	2.65	alcohol dehydrogenase 5 (class III), chi polypeptide
UNK_AA4279	AA442799	22.40	7.47	8.48	5.80	0.0095	2.65	Cluster Incl AA442799: zx65b10.r1 Soares_total_fetus_Nb2HF8_9w Homo sapiens cDNA clone IMAGE:758975 5'. mRNA sequence.
NMP200	AJ131186	23.80	4.36	9.00	8.24	0.0002	2.64	nuclear matrix protein NMP200 related to splicing factor PRP19
LANCL1	Y11395	75.40	16.44	28.54	21.94	0.0006	2.64	LanC (bacterial lantibiotic synthetase component C)-like 1
SIPA1	AB005666	39.00	8.03	14.77	8.09	0.0000	2.64	signal-induced proliferation-associated gene 1
UNK_AL02231;	AL022312	13.00	2.45	4.92	4.05	0.0002	2.64	Cluster Incl AL022312: Human DNA sequence from clone RP5-1104E15 on chromosome 22q12.3-13.1 Contains the MGAT3 gene for mannosyl (beta-1,4-)-gly
RBM4	U89505	13.20	3.70	5.00	4.47	0.0034	2.64	RNA binding motif protein 4
TUBA3	X01703	13.60	3.13	5.15	3.85	0.0010	2.64	Tubulin, alpha, brain-specific
P130	D21262	16.40	5.03	6.23	4.57	0.0061	2.63	nucleolar phosphoprotein p130

TABLE 7

Symbol	Accession							Description
RASGRP2	AI688812	36.00	3.54	13.69	9.99	0.0000	2.63	RAS guanyl releasing protein 2 (calcium and DAG-regulated)
KIAA0746	AB018289	59.20	19.56	22.54	16.44	0.0090	2.63	KIAA0746 protein
HIF1A	U22431	22.00	8.60	6.38	9.33	0.0058	2.62	hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)
KIAA0908	AB020713	47.80	6.83	18.23	13.99	0.0000	2.62	KIAA0908 protein
RAC1	M29870	80.80	8.70	30.85	18.76	0.0000	2.62	ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1)
IRF4	U52682	15.20	3.42	5.85	4.54	0.0009	2.60	interferon regulatory factor 4
MTA1	U35113	11.80	3.83	4.54	3.18	0.0087	2.60	metastasis associated 1
PPIE	AF042386	12.20	2.17	4.69	3.20	0.0001	2.60	peptidylprolyl isomerase E (cyclophilin E)
TNRC11	AF071309	22.80	5.40	8.77	7.14	0.0012	2.60	trinucleotide repeat containing 11 (THR-associated protein, 230 kDa subunit)
YME1L1	AJ132637	60.60	15.71	23.54	22.04	0.0024	2.57	YME1 (S.cerevisiae)-like 1
MCM6	D84557	39.80	7.29	15.48	14.32	0.0003	2.57	minichromosome maintenance deficient (mis5, S. pombe) 6
ZNF91	L11672	19.60	6.02	7.62	5.62	0.0065	2.57	zinc finger protein 91 (HPF7, HTF10)
PTD017	AF072902	35.60	10.01	13.85	9.49	0.0041	2.57	PTD017 protein
KIAA0982	AA152202	68.40	21.28	26.62	17.02	0.0075	2.56	KIAA0982 protein
KIAA0909	AB023199	87.80	10.76	34.23	28.47	0.0000	2.56	KIAA0909 protein
SNRPA	AB020716 M60784	72.20	9.39	28.23	21.55	0.0000	2.56	small nuclear ribonucleoprotein polypeptide A
UNK_AC00438	AC004381	10.80	3.27	4.23	2.98	0.0063	2.55	Cluster incl AC004381: Homo sapiens Chromosome 16 BAC clone CIT987SK-44M2, complete sequence.
ATP6A1	AA056747	73.20	17.81	28.69	17.72	0.0019	2.55	ATPase, H+ transporting, lysosomal (vacuolar proton pump), alpha polypeptide, 70kD, isoform 1
KIAA0922	AB023139	13.60	3.85	5.38	3.12	0.0050	2.53	KIAA0922 protein
HNRPR	AF000364	40.00	3.81	15.65	11.22	0.0000	2.52	heterogeneous nuclear ribonucleoprotein R
ITM2A	AL021786	12.80	4.15	5.08	3.62	0.0092	2.52	integral membrane protein 2A
TCF3	M31523	79.40	16.92	31.54	24.35	0.0007	2.52	transcription factor 3 (E2A immunoglobulin enhancer binding factors E12/E47)
SOS1	L13858	18.00	3.48	7.15	4.78	0.0003	2.52	son of sevenless (Drosophila) homolog 1
AES	AF072902	41.20	11.28	16.33	12.30	0.0035	2.51	amino-terminal enhancer of split
IGKC	M63438	11.60	3.21	4.62	3.20	0.0040	2.51	immunoglobulin kappa constant
KIAA0099	D43951	23.00	3.61	9.15	7.27	0.0001	2.51	KIAA0099 gene product
HT010	AL049948	15.80	3.27	8.31	4.17	0.0006	2.50	uncharacterized hypothalamus protein HT010
CAST	U31346	10.20	2.39	4.08	2.06	0.0019	2.50	calpastatin
D6S81E	Z37166	10.00	2.55	4.00	3.81	0.0025	2.50	MLA-B associated transcript-1
IL2RG	D11086	10.00	3.08	4.00	2.45	0.0078	2.50	interleukin 2 receptor, gamma (severe combined immunodeficiency)
DKFZP434D15	AA447263	14.80	3.63	5.92	4.41	0.0019	2.50	DKFZP434D156 protein
TYK2	X54637	14.80	2.28	5.92	3.71	0.0000	2.50	tyrosine kinase 2
DUSP11	AF023917	19.40	4.04	7.77	5.48	0.0006	2.50	dual specificity phosphatase 11 (RNA/RNP complex 1-interacting)
ATP2A3	Y15724	32.40	6.47	13.00	7.54	0.0005	2.49	ATPase, Ca++ transporting, ubiquitous
ATR	U49844	13.60	3.13	5.46	4.46	0.0013	2.49	ataxia telangiectasia and Rad3 related
UNK_AF05499	AF054996	26.80	3.19	10.77	6.98	0.0000	2.49	Homo sapiens clone 23783 mRNA sequence
GOSR1	AF047438	13.00	2.92	5.23	3.42	0.0011	2.49	golgi SNAP receptor complex member 1
CPSF1	U37012	50.00	6.44	20.15	13.92	0.0000	2.48	cleavage and polyadenylation specific factor 1, 160kD subunit
DDX19	AJ237946	20.20	4.09	8.15	6.88	0.0006	2.48	DEADH (Asp-Glu-Ala-Asp/His) box polypeptide 19 (Dbp5, yeast, homolog)
WBSCR1	D26068	16.00	4.30	6.46	4.58	0.0035	2.48	Williams-Beuren syndrome chromosome region 1
KIAA0164	D79986	23.80	6.30	9.62	7.82	0.0031	2.48	KIAA0164 gene product
UNK_AI74919	AI749193	27.20	5.63	11.00	9.57	0.0007	2.47	Homo sapiens mRNA; cDNA DKFZp586K2123 (from clone DKFZp586K2123)
SIT	AJ010059	76.60	14.31	31.00	16.03	0.0004	2.47	SHP2 interacting transmembrane adaptor
KIAA0999	AB023216	26.20	8.44	10.62	7.04	0.0097	2.47	KIAA0999 protein
EWSR1	X66899	14.60	4.34	5.92	4.03	0.0063	2.46	Ewing sarcoma breakpoint region 1
SIAT9	AB018356	29.20	5.22	11.85	8.74	0.0002	2.46	sialyltransferase 9 (CMP-NeuAc:lactosylceramide alpha-2,3-sialyltransferase; GM3 synthase)
BRD1	AL080149	14.00	2.45	5.69	2.02	0.0004	2.46	bromodomain-containing 1
JUND	X56681	10.40	1.52	4.23	2.77	0.0000	2.46	jun D proto-oncogene
UNK_AL03128	AL031282	20.80	2.17	8.46	6.60	0.0000	2.46	Cluster incl AL031282: Human DNA sequence from clone 283E3 on chromosome 1p36.21-36.33. Contains the alternatively spliced gene for Matrix Metalloprot.
PURA	M96684	10.20	2.77	4.15	2.34	0.0044	2.46	purine-rich element binding protein A
SRM300	AB002322	17.00	5.24	6.92	4.31	0.0081	2.46	RNA binding protein; AT-rich element binding factor
SMARCA4	U29175	13.40	3.65	5.48	5.55	0.0044	2.45	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4
DATF1	AB002331	10.00	3.00	4.08	1.98	0.0081	2.45	death associated transcription factor 1
ARHGEF2	U72206	33.20	3.03	13.54	11.01	0.0000	2.45	rho/rac guanine nucleotide exchange factor (GEF) 2
PPP2R5C	Z69030	13.20	3.96	5.38	3.73	0.0088	2.45	protein phosphatase 2, regulatory subunit B (B56), gamma isoform
NCF1	M55067	22.40	3.21	9.15	5.86	0.0000	2.45	neutrophil cytosolic factor 1 (47kD, chronic granulomatous disease, autosomal 1)
UBA52	AC005253	22.00	5.15	9.00	6.35	0.0015	2.44	ubiquitin A-52 residue ribosomal protein fusion product 1
OIP2	AL050353	57.60	13.87	23.52	16.57	0.0018	2.44	Opa-interacting protein 2
PPP2R2A	M64929	24.00	2.92	9.95	6.45	0.0000	2.44	protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), alpha isoform
NUMA1	Z11584	11.60	2.17	4.65	3.26	0.0003	2.43	nuclear mitotic apparatus protein 1
SSNA1	Z96932	20.60	3.51	8.46	5.71	0.0001	2.43	Sjogren's syndrome nuclear autoantigen 1
KIAA1096	AL099857	34.80	1.30	14.31	9.99	0.0000	2.43	KIAA1096 protein
EIF5A	U17969	11.40	1.52	4.69	3.28	0.0000	2.43	eukaryotic translation initiation factor 5A
TTC3	D63077	37.00	9.30	15.23	16.88	0.0039	2.43	tetratricopeptide repeat domain 3
STAG2	Z75331	14.20	2.86	5.85	5.13	0.0007	2.43	stromal antigen 2
CC1.3	L10910	19.80	3.70	8.15	7.05	0.0005	2.43	splicing factor (CC1.3)
MYC	V00568	19.80	3.27	8.15	6.50	0.0002	2.43	v-myc avian myelocytomatosis viral oncogene homolog
FLJ20479	AI816034	25.40	8.20	10.46	9.25	0.0099	2.43	likely homolog of yeast Nhp2, component of the H/ACA snoRNP; hypothetical protein FLJ20479
SLC25A1	X96924	24.80	2.17	10.23	5.76	0.0000	2.42	solute carrier family 25 (mitochondrial carrier; citrate transporter), member 1

Table 7

Gene	Accession						Ratio	Description
UBE1C	AF046024	11.00	1.58	4.54	2.67	0.0000	2.42	ubiquitin-activating enzyme E1C (homologous to yeast UBA3)
UGP2	U27460	11.00	3.39	4.54	2.30	0.0093	2.42	UDP-glucose pyrophosphorylase 2
HNRPA0	U23803	21.80	6.02	9.00	6.71	0.0043	2.42	heterogeneous nuclear ribonucleoprotein A0
CBF2	M37197	18.00	6.24	6.62	4.27	0.0006	2.42	CCAAT-box-binding transcription factor
RBM5	U23946	58.60	13.52	24.23	16.28	0.0015	2.42	RNA binding motif protein 5
CNIL	AF104398	21.20	4.92	8.77	5.61	0.0018	2.42	cornichon-like
LOC51669	W26659	10.60	1.14	4.38	2.99	0.0000	2.42	HSPC035 protein
KIAA0801	AB018344	63.00	8.60	26.08	16.96	0.0000	2.42	KIAA0801 gene product
SCA2	Y08262	10.40	2.97	4.31	3.01	0.0054	2.41	spinocerebellar ataxia 2 (olivopontocerebellar ataxia 2, autosomal dominant, ataxin 2)
UNK_AL04947	AL04471	10.40	2.70	4.31	1.93	0.0043	2.41	Homo sapiens mRNA; cDNA DKFZp586N012 (from clone DKFZp586N012)
EIF4G1	D12686	20.20	3.90	8.38	6.90	0.0005	2.41	eukaryotic translation initiation factor 4 gamma, 1
POLG	U60325	10.00	2.12	4.15	2.08	0.0011	2.41	polymerase (DNA directed), gamma
GADD45B	AF078077	12.40	2.88	5.15	3.93	0.0016	2.41	growth arrest and DNA-damage-inducible, beta
UNK_X56199	X56199	14.80	2.68	6.15	4.62	0.0003	2.41	Human XIST, coding sequence a mRNA (locus DXS399E)
CSF3R	M59818	12.20	2.49	5.08	3.30	0.0006	2.40	colony stimulating factor 3 receptor (granulocyte)
SLC9A3R1	AF015926	41.00	10.42	17.08	11.42	0.0028	2.40	solute carrier family 9 (sodium/hydrogen exchanger), isoform 3 regulatory factor 1
DKFZP586I2223	AL080214	16.40	2.07	6.85	4.41	0.0000	2.40	DKFZP586I2223 protein
ABCC10	A1004207	11.60	2.51	4.85	3.00	0.0010	2.39	ATP-binding cassette, sub-family C (CFTR/MRP), member 10
UNK_AL08021:AL080218	AL080218	13.80	3.27	5.77	4.53	0.0018	2.39	Homo sapiens mRNA; cDNA DKFZp586N1323 (from clone DKFZp586N1323)
UBE2G2	AF032456	74.60	17.18	31.23	24.26	0.0015	2.39	ubiquitin-conjugating enzyme E2G 2 (homologous to yeast UBC7)
KIAA0546	AB011118	18.00	4.85	7.54	5.16	0.0041	2.39	KIAA0546 protein
PEX10	AA194159	13.40	2.88	5.62	4.31	0.0010	2.39	peroxisome biogenesis factor 10
ADPRT	J03473	17.60	4.83	7.38	6.42	0.0046	2.38	ADP-ribosyltransferase (NAD+; poly (ADP-ribose) polymerase)
LOC51165	Z77324	19.60	3.91	8.23	5.07	0.0008	2.38	SH3 domain-containing protein 6511
HNRPDL	D89678	23.60	3.05	9.92	7.08	0.0000	2.38	heterogeneous nuclear ribonucleoprotein D-like
DKFZP586F13	AL050373	27.60	7.54	11.62	8.10	0.0044	2.38	hypothetical protein
RAD23A	D21235	25.40	2.97	10.69	6.84	0.0002	2.38	RAD23 (S. cerevisiae) homolog A
PPP1CA	S57501	169.20	27.80	71.23	38.15	0.0001	2.38	protein phosphatase 1, catalytic subunit, alpha isoform
RANBP2L1	AF012086	23.00	3.94	9.69	7.59	0.0003	2.37	RAN binding protein 2-like 1
SAFB	U72355	10.40	2.30	4.38	3.73	0.0014	2.37	scaffold attachment factor B
KIAA0082	D43949	67.60	14.88	28.54	18.19	0.0012	2.37	KIAA0082 protein
LOC51580	D14041	18.00	4.80	7.62	5.36	0.0039	2.36	H2K binding factor-2
PDHB	D90086	21.80	3.70	9.23	8.40	0.0005	2.36	pyruvate dehydrogenase (lipoamide) beta
KIAA0187	D80009	15.80	4.32	6.69	4.52	0.0047	2.36	KIAA0187 gene product
PSMD3	D67025	15.80	1.30	6.69	4.48	0.0000	2.36	proteasome (prosome, macropain) 26S subunit, non-ATPase, 3
DKFZP564M24	AL080119	11.80	3.19	5.00	3.42	0.0044	2.36	DKFZP564M2423 protein
MYCL2	J03069	11.80	2.17	5.00	4.47	0.0006	2.36	v-myc avian myelocytomatosis viral oncogene homolog 2
HERC2	AF041080	31.40	7.70	13.31	9.20	0.0024	2.36	hect domain and RLD 2
UNK_U79273	U79273	13.60	1.14	5.77	4.46	0.0000	2.36	Human clone 23933 mRNA sequence
KIAA1055	AB020978	17.40	3.65	7.38	5.80	0.0009	2.36	KIAA1055 protein
FLJ20259	W27545	15.40	3.78	6.54	4.89	0.0024	2.36	hypothetical protein FLJ20259
KIAA0252	D87440	13.40	3.78	5.69	3.86	0.0056	2.35	KIAA0252 protein
ISG15	AA203213	15.20	4.60	6.46	5.16	0.0080	2.35	interferon-stimulated protein, 15 kDa
DKFZP564A12	W26496	24.60	6.07	10.46	6.01	0.0028	2.35	DKFZp564A122 protein
DKFZP564M24	AL080119	17.00	3.67	7.23	4.59	0.0011	2.35	DKFZP564M2423 protein
PABPC4	U75686	34.00	5.70	14.46	8.72	0.0002	2.35	poly(A)-binding protein, cytoplasmic 4 (inducible (form)
SIP2-28	U85611	30.00	2.45	12.77	7.53	0.0009	2.35	calcium and integrin binding protein (DNA-dependent protein kinase interacting protein)
DUT	U31930	13.00	2.74	5.54	4.03	0.0009	2.35	dUTP pyrophosphatase
TIAL1	D64015	22.20	4.92	9.46	5.44	0.0014	2.35	TIA1 cytotoxic granule-associated RNA-binding protein-like 1
DHPS	U26266	20.20	6.02	8.62	4.84	0.0081	2.34	deoxyhypusine synthase
GADD34	U83981	22.00	8.20	9.33	7.35	0.0056	2.34	growth arrest and DNA-damage-inducible 34
KIAA0788	AB016331	11.00	2.35	4.69	3.04	0.0010	2.34	KIAA0789 protein
UNK_AJ22487:AJ224875	L40397	40.20	5.22	17.15	13.63	0.0039	2.34	Homo sapiens mRNA for putative glucosyltransferase, partial cds
TMP21	AB028989	10.80	2.17	4.62	2.84	0.0001	2.34	transmembrane trafficking protein
MAPK8IP3	AL044951	16.20	3.70	6.92	4.27	0.0006	2.34	mitogen-activated protein kinase 8 interacting protein 3
UNK_AL04995	AB011149	28.60	8.99	12.23	8.53	0.0016	2.34	Homo sapiens mRNA; cDNA DKFZp564O0122 (from clone DKFZp564O0122)
DDX16	L20046	10.60	2.07	4.54	2.99	0.0099	2.34	DEADH (Asp-Glu-Ala-Asp/His) box polypeptide 16
ERCC5	L36696	10.60	1.67	4.54	2.76	0.0005	2.34	excision repair cross-complementing rodent repair deficiency, complementation group 5 (xeroderma pigmentosum, complementation group G (Cockayne synd...
RALY	L20859	13.80	3.49	5.92	4.41	0.0001	2.34	RNA-binding protein (autoantigenic)
SLC20A1	AB029003	59.60	3.78	25.62	17.28	0.0031	2.33	solute carrier family 20 (phosphate transporter), member 1
KIAA1080	U47101	13.60	3.65	5.85	4.10	0.0000	2.33	KIAA1080 protein; Golgi-associated, gamma-adaptin ear containing, ARF-binding protein 2
NIFU	AF060219	16.80	3.42	7.23	4.34	0.0043	2.33	nitrogen fixation cluster-like
CHC1L	AF032885	26.80	4.92	11.54	8.52	0.0008	2.32	chromosome condensation 1-like
FOXO1A	D89937	53.60	14.84	23.08	21.23	0.0004	2.32	forkhead box O1A (rhabdomyosarcoma)
FSTL1	AL049450	13.40	3.85	5.77	3.59	0.0058	2.32	follistatin-like 1
UNK_AL04945:AL04950	U02570	16.60	5.13	7.15	5.84	0.0066	2.32	Homo sapiens mRNA; cDNA DKFZp586B1922 (from clone DKFZp586B1922)
ARHGAP1	U04953	11.60	2.70	5.00	3.49	0.0094	2.32	Rho GTPase activating protein 1
IARS	U04953	11.60	2.07	5.00	3.58	0.0019	2.32	isoleucine-tRNA synthetase
PRKCD	D10495	11.60	2.07	5.00	3.58	0.0003	2.32	protein kinase C, delta

Table 7

Symbol	Accession					p-value	Ratio / Description
RNF3	W25793	14.80	3.70	6.38	4.05	0.0030	2.32 ring finger protein 3
TRIM	AJ224878	55.40	15.88	23.92	21.38	0.0068	2.32 T-cell receptor interacting molecule
UNK_N25547	N25547	22.80	5.89	9.85	8.47	0.0039	2.32 Homo sapiens PAK2 mRNA, complete cds
DIPA	U53325	48.80	8.78	21.08	16.76	0.0004	2.32 hepatitis delta antigen-interacting protein A
IGL@	X57809	13.00	2.92	6.82	4.23	0.0015	2.32 immunoglobulin lambda locus
NARS	D84273	122.40	27.81	52.92	47.83	0.0022	2.31 asparaginyl-tRNA synthetase
RBMS1P	D82351	14.40	1.34	6.23	4.38	0.0022	2.31 RNA binding motif, single stranded interacting protein 1, pseudogene
NFATC3	L41067	31.40	6.50	13.82	10.18	0.0009	2.31 nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3
KNS2	L04733	53.00	11.34	23.00	16.56	0.0009	2.30 kinesin 2 (60-70kD)
RGS2	L13463	23.00	2.45	10.00	6.71	0.0011	2.30 regulator of G-protein signalling 2, 24kD
KIAA0682	AB014582	44.40	10.06	19.31	11.39	0.0000	2.30 KIAA0682 gene product
RING1	Z14000	21.40	3.36	9.31	6.13	0.0017	2.30 ring finger protein 1
PIGH	L19783	12.20	1.30	5.31	3.71	0.0001	2.30 phosphatidylinositol glycan, class H
COPEB	AF001461	15.20	4.15	6.62	5.03	0.0000	2.30 core promoter element binding protein
KIAA0346	AB002344	42.40	9.07	18.46	12.86	0.0051	2.30 KIAA0346 protein
PRKRI	AI095508	16.60	3.65	7.23	4.19	0.0011	2.30 protein-kinase, interferon-inducible double stranded RNA dependent inhibitor
ALOX5AP	A1806222	97.00	23.16	42.31	28.04	0.0020	2.29 arachidonate 5-lipoxygenase-activating protein
FCGR2B	M28696	25.20	6.18	11.00	8.36	0.0023	2.29 Fc (fragment of IgG, low affinity IIb, receptor for (CD32)
CD2	M16336	16.20	3.03	7.08	5.82	0.0028	2.29 CD2 antigen (p50), sheep red blood cell receptor
NK4	AA631972	19.00	5.10	8.31	6.32	0.0007	2.29 natural killer cell transcript 4
VAMP2	AL050223	14.60	2.51	6.38	4.35	0.0047	2.29 vesicle-associated membrane protein 2 (synaptobrevin 2)
A2LP	U70671	10.20	2.28	4.46	3.43	0.0003	2.29 ataxin 2 related protein
SMARCA2	D26155	46.40	11.63	20.31	13.36	0.0017	2.28 SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2
KI-1/57	U77327	21.60	3.21	9.46	5.98	0.0032	2.28 intracellular antigen detected by monoclonal antibody Ki-1; intracellular hyaluronan-binding protein
KIAA0436	AB007896	28.80	6.39	12.54	9.58	0.0001	2.28 putative L-type neutral amino acid transporter
MBD4	AF072250	21.40	5.81	9.38	6.70	0.0017	2.28 methyl-CpG binding domain protein 4
KIAA0737	AB018280	11.40	1.52	5.00	3.85	0.0051	2.28 KIAA0737 gene product
TIF1B	X97548	11.40	2.70	5.00	3.85	0.0001	2.28 KRAB-associated protein 1
CBX1	U35451	21.20	5.26	9.31	6.81	0.0024	2.28 chromobox homolog 1 (Drosophila HP1 beta)
HSPC004	AI827793	46.60	8.76	20.46	14.33	0.0031	2.28 hypothetical protein
HADH2	AF035555	22.40	5.27	9.85	7.87	0.0005	2.28 hydroxyacyl-Coenzyme A dehydrogenase, type II
IL10RA	U00672	12.60	3.05	5.54	4.18	0.0024	2.28 interleukin 10 receptor, alpha
CHD3	U91543	36.20	3.98	15.92	11.35	0.0027	2.27 chromodomain helicase DNA binding protein 3
BUB3	AF047472	26.40	5.13	11.62	8.57	0.0000	2.27 BUB3 (budding uninhibited by benzimidazoles 3, yeast) homolog
NCOR2	U37146	16.80	3.97	7.31	4.63	0.0058	2.27 nuclear receptor co-repressor 2
GILZ	AI615895	13.80	2.05	6.08	4.55	0.0025	2.27 glucocorticoid-induced leucine zipper
POLR2B	X63563	13.80	3.56	6.08	3.59	0.0002	2.27 polymerase (RNA) II (DNA directed) polypeptide B (140kD)
DNM2	L36983	22.00	6.52	9.69	8.22	0.0041	2.27 dynamin 2
P23	L24804	22.00	4.12	9.89	7.65	0.0096	2.27 unactive progesterone receptor, 23 kD
ZYX	X95735	11.00	1.22	4.85	2.97	0.0007	2.27 zyxin
PPP1R2	U6B111	30.00	6.69	13.23	9.45	0.0000	2.27 protein phosphatase 1, regulatory (inhibitor) subunit 2
SEC24B	AJ131245	15.00	1.87	6.62	5.20	0.0019	2.27 SEC24 (S. cerevisiae) related gene family, member B
UNK_L29376	L29376	18.00	3.54	8.38	6.56	0.0001	2.27 Homo sapiens (clone 3.8-1) MHC class I mRNA fragment
CEBPB	X52560	17.60	3.21	7.77	5.66	0.0006	2.27 CCAAT/enhancer binding protein (C/EBP), beta
RBL2	X74594	13.40	2.70	5.92	4.11	0.0005	2.26 retinoblastoma-like 2 (p130)
PRPSAP1	D61391	12.00	2.35	5.77	3.73	0.0008	2.26 phosphoribosyl pyrophosphate synthetase-associated protein 1
KIAA0191	D83776	36.00	8.04	15.92	12.19	0.0007	2.26 KIAA0191 protein
NRG1	L12260	11.60	2.70	5.15	3.26	0.0074	2.26 neuregulin 1
POLR2C	J05448	11.60	2.70	5.15	3.39	0.0026	2.26 polymerase (RNA) II (DNA directed) polypeptide C (33kD)
TIEG	S81439	78.60	18.50	35.48	26.69	0.0053	2.26 TGFB inducible early growth response
H-PLK	M55422	34.60	10.29	15.38	11.05	0.0097	2.26 Kruppel-related zinc finger protein
CDC16	U18291	39.40	11.33	17.46	13.25	0.0072	2.26 CDC16 (cell division cycle 16, S. cerevisiae, homolog)
TRA@	M12959	53.60	5.68	23.77	17.62	0.0001	2.26 T cell receptor alpha locus
CEBPD	M83667	14.00	2.74	6.23	3.24	0.0004	2.25 CCAAT/enhancer binding protein (C/EBP), delta
STAT2	U18671	45.80	7.56	20.38	14.13	0.0007	2.25 signal transducer and activator of transcription 2, 113kD
UNK_D28423	D28423	21.60	5.46	9.62	4.84	0.0002	2.25 Cluster incl D28423: Human mRNA for pre-mRNA splicing factor SRp20, 5UTR (sequence from the 5'cap to the start codon).
KIAA0594	AB011166	26.80	7.44	11.85	7.82	0.0041	2.25 KIAA0594 protein
UNK_AI659108	AI659108	50.60	6.11	22.54	15.50	0.0064	2.25 Homo sapiens chromosome 19, cosmid R26379
IL17R	U58917	13.00	3.61	5.77	4.46	0.0061	2.25 interleukin 17 receptor
UNK_U53588	U53588	31.20	8.04	13.85	9.61	0.0040	2.25 Cluster incl U53588: Homo sapiens MHC class 1 region.
NXF1	AJ132712	19.20	3.35	8.54	6.39	0.0004	2.25 nuclear RNA export factor 1
UNK_W73822	W73822	10.20	1.79	4.54	3.31	0.0004	2.25 Cluster incl W73822: zd52f10.s1 Soares_fetal_heart_NbHH19W Homo sapiens cDNA clone IMAGE:344299 3'. mRNA sequence.
CLK2	L29218	14.00	2.74	6.23	3.24	0.0007	2.25 CDC-like kinase 2
UNK_AI743654	AI743654	45.80	7.56	20.38	14.13	0.0002	2.25 Homo sapiens cDNA FLJ11585 fis, clone HEMBA1003692
STK17A	AI861743	21.60	5.46	9.62	4.84	0.0041	2.25 serine/threonine kinase 17a (apoptosis-inducing)
KIAA0700	AB014600	26.80	7.44	11.85	7.82	0.0064	2.25 KIAA0700 protein
POU2AF1	Z49194	50.60	6.11	22.54	15.50	0.0000	2.25 POU domain, class 2, associating factor 1
CDC25B	S78187	29.00	3.32	12.92	9.00	0.0000	2.24 cell division cycle 25B
SKIV2L	U09877	29.00	3.63	12.82	8.45	0.0008	2.24 superkiller viralicidic activity 2 (S. cerevisiae homolog)-like

Table 7

Symbol	Accession						Description
KIAA0169	D79991	12.60	2.07	5.62	4.13	0.0003	2.24 KIAA0169 protein
SOD1	X02317	21.40	5.03	9.54	7.10	0.0024	2.24 superoxide dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))
STAM	U43899	27.60	5.50	12.31	11.89	0.0021	2.24 signal transducing adaptor molecule (SH3 domain and ITAM motif) 1
HNRPM	L03532	30.80	8.76	15.08	10.24	0.0042	2.24 heterogeneous nuclear ribonucleoprotein M
CDC2L1	M37712	25.00	7.48	11.15	6.35	0.0095	2.24 cell division cycle 2-like 1 (PITSLRE proteins)
GALT	M60091	10.00	2.92	4.46	2.47	0.0085	2.24 galactose-1-phosphate uridylyltransferase
KATNB1	AF052432	21.20	4.27	9.46	6.09	0.0008	2.24 katanin p80 (WD40-containing) subunit B 1
IGHM	X59529	18.20	2.68	7.23	4.85	0.0002	2.24 immunoglobulin heavy constant mu
TRN-SR	AA192359	21.00	5.15	9.38	6.40	0.0031	2.24 transportin-SR
NHP2L1	Z63340	58.00	10.20	25.92	19.73	0.0005	2.24 non-histone chromosome protein 2 (S. cerevisiae)-like 1
FYB	AF001862	11.00	1.87	4.92	2.66	0.0002	2.23 FYN-binding protein (FYB-120/130)
KIAA0174	D79996	11.00	3.32	4.92	3.01	0.0098	2.23 KIAA0174 gene product
UNK_AL031775:AL031775		11.00	1.87	4.92	2.29	0.0003	2.23 Cluster Incl AL031775: Human DNA sequence from clone 30M3 on chromosome 6p22.1-22.3. Contains three novel genes, one similar to C. elegans Y61D3A.4
ZAP70	L05148	11.00	2.83	4.92	3.48	0.0041	2.23 zeta-chain (TCR) associated protein kinase (70 kD)
CHES1	U68723	13.40	2.51	6.00	4.60	0.0007	2.23 checkpoint suppressor 1
UNK_AA780049 AA780049	AB029020	13.40	2.51	6.00	3.76	0.0005	2.23 Homo sapiens cDNA: FLJ21439 fis, clone COL04352
KIAA1097	AB029020	52.20	4.76	23.38	13.91	0.0000	2.23 KIAA1097 protein
ATP5A1	D14710	19.40	4.83	8.69	6.09	0.0034	2.23 ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1, cardiac muscle
BAX	U19599	21.80	3.56	9.77	7.13	0.0003	2.23 BCL2-associated X protein
ARRB2	AF106941	10.80	1.48	4.85	2.34	0.0000	2.23 arrestin, beta 2
ATM	U26455	18.00	5.29	8.08	5.85	0.0085	2.23 ataxia telangiectasia mutated (includes complementation groups A, C and D)
CA150	AF017789	14.40	2.70	6.46	4.88	0.0007	2.23 transcription factor CA150
MOF	AI417075	19.20	1.79	8.62	4.17	0.0000	2.23 member of MYST family histone acetyl transferases, homolog of Drosophila MOF
NPIP	AC002045	15.60	4.72	7.00	4.34	0.0100	2.23 nuclear pore complex interacting protein
UNK_AF026816:AF026816	AB014547	14.40	3.51	6.46	4.70	0.0031	2.23 Homo sapiens putative oncogene protein mRNA, partial cds
MTMR4	AB014547	24.00	3.32	10.77	7.78	0.0001	2.23 myotubularin related protein 4
SF3A3	X81789	13.20	3.11	5.92	4.70	0.0028	2.23 splicing factor 3a, subunit 3, 60kD
PIGC	D85418	43.00	10.68	19.31	10.51	0.0036	2.23 phosphatidylinositol glycan, class C
KIAA0349	AB002347	41.60	5.86	18.69	12.15	0.0001	2.23 KIAA0349 protein
ZNF198	AJ224901	16.60	3.51	7.46	5.17	0.0013	2.22 zinc finger protein 198
TCF8	D15050	15.40	3.44	6.92	4.07	0.0018	2.22 transcription factor 8 (represses interleukin 2 expression)
AP1G2	AI741633	13.00	3.39	5.85	4.24	0.0046	2.22 adaptor-related protein complex 1, gamma 2 subunit
PTPRE	X54134	23.60	6.58	10.62	7.77	0.0066	2.22 protein tyrosine phosphatase, receptor type, E
VHL	L15409	56.60	3.85	25.46	16.16	0.0000	2.22 von Hippel-Lindau syndrome
AP3D1	AF002163	10.60	2.51	4.77	3.72	0.0028	2.22 adaptor-related protein complex 3, delta 1 subunit
HCDI	AH49623	25.80	3.80	11.62	9.20	0.0003	2.22 HCDI protein
MGP	W28510	14.00	2.45	6.31	4.71	0.0005	2.22 matrix Gla protein
IK	AJ005579	60.60	11.15	27.31	21.19	0.0007	2.22 IK cytokine, down-regulator of HLA II
KIAA1028	AB028951	16.80	4.27	8.92	5.68	0.0014	2.22 KIAA1028 protein
LYL1	M22637	32.60	6.11	14.69	7.09	0.0006	2.22 lymphoblastic leukemia derived sequence 1
STK10	AB015718	12.80	2.49	5.77	4.13	0.0016	2.22 serine/threonine kinase 10
DKFZP566H07	AL050060	50.00	10.84	22.54	17.91	0.0018	2.22 DKFZP566H073 protein
KIAA0515	AB011087	18.60	5.41	8.38	5.52	0.0083	2.22 KIAA0515 protein
USP1	AB014458	16.20	4.38	7.31	5.25	0.0056	2.22 ubiquitin specific protease 1
ARIH1	AJ009771	15.00	2.83	6.77	4.78	0.0007	2.22 ariadne (Drosophila) homolog, ubiquitin-conjugating enzyme E2-binding protein. 1
UNK_W26634 W26634	W26634	30.00	5.15	13.54	7.21	0.0003	2.22 Cluster Incl W26634: 34b10 Human retina cDNA randomly primed sublibrary Homo sapiens cDNA, mRNA sequence.
ARHGDIA	X69550	48.20	4.09	21.77	17.28	0.0001	2.21 Rho GDP dissociation inhibitor (GDI) alpha
HDAC1	U50079	12.60	3.51	5.69	4.19	0.0067	2.21 histone deacetylase 1
XIP	AF029890	12.60	2.30	5.69	3.88	0.0005	2.21 hepatitis B virus x-interacting protein (9.6kD)
KIAA0365	AB002383	22.80	3.77	10.31	6.51	0.0002	2.21 KIAA0365 gene product
PHF2	AB023143	49.00	13.40	22.15	16.12	0.0061	2.21 PHD finger protein 2
KIAA0544	AB011116	14.80	3.77	6.69	4.44	0.0040	2.21 KIAA0544 protein
ADARB1	U76421	31.80	5.22	14.38	8.53	0.0092	2.21 adenosine deaminase, RNA-specific, B1 (homolog of rat RED1)
NCP	X59408	31.80	6.69	14.38	10.60	0.0014	2.21 membrane cofactor protein (CD46, trophoblast-lymphocyte cross-reactive antigen)
KIAA0117	AA887460	10.20	2.39	4.62	3.12	0.0025	2.21 KIAA0117 protein
SFRS6	AB031681	17.00	1.87	7.69	5.78	0.0001	2.21 splicing factor, arginine/serine-rich 6
KIAA0926	AB023143	26.00	4.64	11.77	8.79	0.0006	2.21 KIAA0926 protein
ITPR3	U01062	15.80	3.03	7.15	4.08	0.0006	2.21 inositol 1,4,5-triphosphate receptor, type 3
EIF5	AL080102	12.40	3.65	5.62	3.20	0.0001	2.21 eukaryotic translation initiation factor 5
NCL	M60858	49.60	6.99	22.46	14.28	0.0001	2.21 nucleolin
RASA1	M23379	14.60	3.21	6.62	4.05	0.0017	2.21 RAS p21 protein activator (GTPase activating protein) 1
PES1	U78310	16.80	3.70	7.62	4.91	0.0017	2.21 pescadillo (zebrafish) homolog 1, containing BRCT domain
RAD21	D38551	24.60	4.39	11.15	6.57	0.0004	2.21 RAD21 (S. pombe) homolog
KIAA1041	AB028964	15.60	2.51	7.08	3.28	0.0002	2.20 KIAA1041 protein
ETR101	M62831	33.40	7.83	15.15	12.03	0.0029	2.20 immediate early protein
E2F4	AA552140	17.80	3.03	8.08	5.44	0.0003	2.20 E2F transcription factor 4, p107/p130-binding
DBP	U48213	22.20	4.55	10.08	11.03	0.0046	2.20 D site of albumin promoter (albumin D-box) binding protein
TEB4	AB011169	14.40	3.58	6.54	3.69	0.0037	2.20 similar to S. cerevisiae SSM4
KIAA1067	AB028990	75.20	12.60	34.15	23.68	0.0003	2.20 KIAA1067 protein

Table 7

Symbol	Accession	V1	V2	V3	V4	P-value	Ratio	Description
SF3B2	U1371	63.00	28.62	9.67	25.14	0.0007	2.20	splicing factor 3b, subunit 2, 145kD
DGSI	L77566	11.00	5.00	1.22	2.16	0.0000	2.20	DiGeorge syndrome critical region gene DGSI
DUSP1	X68277	17.60	8.00	1.67	4.64	0.0000	2.20	dual specificity phosphatase 1
KIAA0911	AB020718	11.00	5.00	2.12	3.79	0.0009	2.20	KIAA0911 protein
RABIF	U74324	27.40	12.46	7.96	9.00	0.0085	2.20	RAB interacting factor
KIAA1111	AB029034	20.80	9.46	3.56	6.79	0.0004	2.20	KIAA1111 protein
MAPRE2	X94232	14.20	6.46	3.03	4.99	0.0017	2.20	microtubule-associated protein, RP/EB family, member 2
GRSF1	U07231	24.00	10.92	4.64	7.94	0.0009	2.20	G-rich RNA sequence binding factor 1
KIAA0752	AB016295	12.00	5.46	3.39	3.99	0.0074	2.20	KIAA0752 protein
PPP2R4	X73478	13.00	5.92	2.55	3.88	0.0008	2.19	protein phosphatase 2A, regulatory subunit B' (PR 53)
CFDP1	AB009285	31.40	14.31	5.13	9.01	0.0002	2.19	craniofacial development protein 1
DED	U51698	31.40	14.31	8.14	9.74	0.0047	2.19	apoptosis antagonizing transcription factor
PSMB7	D38048	23.80	10.85	6.50	6.18	0.0064	2.19	proteasome (prosome, macropain) subunit, beta type, 7
UNK_W32483	W32483	16.20	7.38	3.96	5.64	0.0035	2.19	Human DNA sequence from clone 283E3 on chromosome 1p36.21-36.33. Contains the alternatively spliced gene for Matrix Metalloproteinase in the Female R...
CPT1B	V00663	30.20	13.77	7.29	9.99	0.0032	2.19	carnitine palmitoyltransferase I, muscle
ERPROT213-2	U94636	19.40	8.85	4.72	5.49	0.0032	2.19	protein with polyglutamine repeat; calcium (ca2+) homeostasis endoplasmic reticulum protein
UNK_AL02210	AL022101	19.40	8.85	5.50	6.47	0.0077	2.19	Cluster Incl AL022101: Homo sapiens DNA sequence from PAC 845O24 on chromosome 1p36.1-36.2. Contains a gene for a Heterogenous Nuclear Ribonucle...
MADH3	U68019	17.20	7.85	3.11	6.43	0.0009	2.19	MAD (mothers against decapentaplegic, Drosophila) homolog 3
CCT8	D13627	42.80	19.54	3.56	12.86	0.0000	2.19	chaperonin containing TCP1, subunit 8 (theta)
TIM17	X97544	74.80	34.15	17.54	23.54	0.0027	2.19	translocase of inner mitochondrial membrane 17 (yeast) homolog A
DKFZP564O24	AL050015	12.80	5.85	3.63	3.53	0.0078	2.19	DKFZP564O243 protein
GNG10	U31383	12.80	5.85	3.03	4.45	0.0030	2.19	guanine nucleotide binding protein 10
KIAA0073	D38552	22.40	10.23	3.13	7.73	0.0002	2.19	KIAA0073 protein
SFRS10	U88063	12.80	5.85	2.39	3.72	0.0006	2.19	splicing factor, arginine/serine-rich (transformer 2 Drosophila homolog) 10
GATA3	X56072	38.20	17.46	6.83	13.54	0.0007	2.19	GATA-binding protein 3
RRN3	AF001549	28.60	13.08	7.02	9.95	0.0037	2.19	RNA polymerase I transcription factor RRN3
PPIE	AF042385	71.80	32.85	12.36	26.29	0.0007	2.19	peptidylprolyl isomerase E (cyclophilin E)
SATB1	M97287	19.00	8.69	4.00	3.99	0.0016	2.19	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)
DKFZP586G01	AL050126	185.60	84.92	39.66	45.01	0.0015	2.19	DKFZP586G011 protein
IMPDH1	J05272	62.20	28.46	15.25	26.51	0.0051	2.19	IMP (inosine monophosphate) dehydrogenase 1
PIG7	AF010312	55.80	25.54	13.05	17.76	0.0027	2.18	LPS-induced TNF-alpha factor
ARHH	Z35227	12.60	5.77	2.30	3.52	0.0005	2.18	res homolog gene family, member H
DEK	X64229	16.80	7.69	3.11	6.22	0.0010	2.18	DEK oncogene (DNA binding)
HSU53209	U53209	17.80	8.15	2.59	4.69	0.0001	2.18	transformer-2 alpha (htra-2 alpha)
KIAA0554	AB011126	52.20	23.92	11.82	16.21	0.0022	2.18	KIAA0554 protein
BIRC3	U45878	24.00	11.00	4.00	7.87	0.0004	2.18	baculoviral IAP repeat-containing 3
TRB@	X00437	65.60	30.08	18.34	23.73	0.0076	2.18	T cell receptor beta locus
LGALS8	L78132	10.40	4.77	2.19	3.24	0.0014	2.18	lectin, galactoside-binding, soluble, 8 (galectin 8)
SPN	AF049460	26.00	11.92	7.52	7.86	0.0086	2.18	suppressin (nuclear deformed epidermal autoregulatory factor-1 (DEAF-1)-related)
BAF53A	AF041474	43.20	19.85	8.61	12.34	0.0009	2.18	BAF53
NAP1L1	M66667	14.40	6.62	2.61	4.09	0.0005	2.18	nucleosome assembly protein 1-like 1
SSH3BP1	AF006516	44.20	20.31	6.76	16.79	0.0006	2.18	spectrin SH3 domain binding protein 1
FUS1	AF055479	122.20	56.23	23.75	33.99	0.0008	2.17	lung cancer candidate
M11S1	Z40842	11.20	5.15	1.64	2.61	0.0001	2.17	membrane component, chromosome 11, surface marker 1
TRF4	AB005754	11.20	5.15	2.28	3.36	0.0011	2.17	topoisomerase-related function protein 4-1
EVI2B	M60030	24.40	11.23	6.23	7.08	0.0045	2.17	ecotropic viral integration site 2B
PPIE	AF042386	13.20	6.08	3.03	4.54	0.0027	2.17	peptidylprolyl isomerase E (cyclophilin E)
UNK_AI808712	AI808712	14.20	6.54	2.28	4.27	0.0002	2.17	Cluster Incl AI808712: w57c05.x1 Soares_NFL_T_GBC_S1 Homo sapiens cDNA clone IMAGE:2359688 3', mRNA sequence.
AP2B1	M34175	33.40	15.38	7.09	10.67	0.0016	2.17	adaptor-related protein complex 2, beta 1 subunit
PTPRCAP	X97267	33.40	15.38	4.16	8.49	0.0000	2.17	protein tyrosine phosphatase, receptor type, C-associated protein
PSMD9	AI347155	35.40	16.31	8.08	13.73	0.0032	2.17	proteasome (prosome, macropain) 26S subunit, non-ATPase, 9
SFRS2	X75755	20.20	9.31	3.96	5.79	0.0009	2.17	splicing factor, arginine/serine-rich 2
CHKL	AL096780	21.20	9.77	5.36	7.32	0.0045	2.17	choline kinase-like
KIAA0128	AI819942	10.00	4.62	1.41	2.47	0.0001	2.17	KIAA0128 protein; septin 2
SF3A2	L21990	20.00	9.23	3.67	6.73	0.0008	2.17	splicing factor 3a, subunit 2, 66kD
AIM1	U83115	16.00	7.38	4.06	4.65	0.0044	2.17	absent in melanoma 1
CTBP1	U37408	13.00	6.00	3.54	4.93	0.0071	2.17	C-terminal binding protein 1
SYPL	X86194	12.00	5.54	1.87	3.43	0.0002	2.17	synaptophysin-like protein
TMEM4	AB015631	12.00	5.54	2.12	3.71	0.0005	2.17	transmembrane protein 4
UNK_AF07053	AF070535	14.00	6.46	2.92	4.07	0.0013	2.17	Homo sapiens clone 24432 mRNA sequence
KIAA0618	N29685	29.80	13.77	4.44	8.28	0.0001	2.16	KIAA0618 gene product
NRD1	X93209	27.80	12.85	3.96	7.02	0.0001	2.16	nardilysin (N-arginine dibasic convertase)
SNRPF	AI032612	24.80	11.46	6.38	8.53	0.0050	2.16	small nuclear ribonucleoprotein polypeptide F
UNK_AL04112	AL041124	21.80	10.08	5.89	5.79	0.0054	2.16	Cluster Incl AL04112: DKFZp434D0316_s1 434 (synonym: htes3) Homo sapiens cDNA clone DKFZp434D0316 3'; mRNA sequence.
NP220	D83032	14.80	6.85	2.49	4.34	0.0003	2.16	nuclear protein
ZNF216	AF062346	14.80	6.85	3.27	4.47	0.0020	2.16	zinc finger protein 216
TNFRSF10B	AF016266	26.60	12.31	5.77	8.68	0.0019	2.16	tumor necrosis factor receptor superfamily, member 10b
ARHGDIB	X69549	35.40	16.38	6.27	11.14	0.0005	2.16	Rho GDP dissociation inhibitor (GDI) beta
BCL6	U00115	11.80	5.46	3.03	3.69	0.0048	2.16	B-cell CLL/lymphoma 6 (zinc finger protein 51)

Table 7

Gene	Accession					p-value	Description
H1FX	D64112	23.60	4.34	10.92	6.55	0.0005	2.16 H1 histone family, member X
SF3B1	AF054284	11.80	2.39	5.46	4.14	0.0014	2.16 splicing factor 3b, subunit 1, 155kD
ETFA	J04058	34.20	9.60	15.85	13.19	0.0065	2.16 electron-transfer-flavoprotein, alpha polypeptide (glutaric aciduria II)
TIC	U63127	24.40	4.72	11.31	7.45	0.0009	2.16 SEC7 homolog
ADAR	X79448	81.00	17.71	37.54	26.80	0.0020	2.16 adenosine deaminase, RNA-specific
TRIP3	L40410	13.60	3.85	6.31	4.91	0.0084	2.16 thyroid hormone receptor interactor 3
LCP2	U20158	11.60	2.19	5.38	3.84	0.0009	2.15 lymphocyte cytosolic protein 2 (SH2 domain-containing leukocyte protein of 76kD)
KIAA0242	D87894	87.60	25.42	40.77	29.33	0.0092	2.15 KIAA0242 protein
HSPA8	Y00371	10.60	2.41	4.92	3.23	0.0024	2.15 heat shock 70kD protein 8
FMNL	AJ008112	25.00	4.30	11.82	9.40	0.0009	2.15 formin-like
LY75	AF011333	86.40	16.68	40.15	27.88	0.0009	2.15 lymphocyte antigen 75
IFITM3	X57352	19.20	5.02	8.92	8.30	0.0075	2.15 interferon induced transmembrane protein 3 (1-8U)
ILF3	U10324	42.20	6.76	19.62	14.49	0.0004	2.15 interleukin enhancer binding factor 3, 90kD
MAP3K4	AF002715	18.20	4.49	8.46	5.61	0.0039	2.15 mitogen-activated protein kinase kinase kinase 4
KIAA0442	AB007902	16.20	2.68	7.54	4.52	0.0003	2.15 KIAA0442 protein
COX11	U79270	14.20	3.63	6.62	4.13	0.0048	2.15 COX11 (yeast) homolog, cytochrome c oxidase assembly protein
RUNX3	Z35278	12.20	2.95	5.69	4.79	0.0045	2.14 runt-related transcription factor 3
UNK_AL05014	AL050148	26.20	5.72	12.23	8.32	0.0020	2.14 Homo sapiens clone 25129 mRNA sequence
UNK_W25986	W25986	16.80	2.59	7.85	5.34	0.0003	2.14 Homo sapiens cDNA FLJ20532 fis, clone KAT10877
SORL1	Y08110	36.40	2.88	17.00	9.33	0.0000	2.14 sortilin-related receptor, L(DLR class) A repeats-containing
KIAA0138	D50928	66.80	19.38	31.23	23.58	0.0098	2.14 KIAA0138 gene product
DIM1	AF023612	10.20	2.39	4.77	2.92	0.0029	2.14 similar to S. pombe dim1+
LOC51317	N98687	10.20	2.28	4.77	3.79	0.0028	2.14 hypothetical protein
TAF2I	X83928	32.40	4.28	15.15	7.70	0.0000	2.14 TATA box binding protein (TBP)-associated factor, RNA polymerase II, I, 23kD
KIAA0766	AB018309	14.80	3.11	6.92	5.58	0.0022	2.14 KIAA0766 gene product
SPTLC1	Y08685	19.40	3.58	9.08	6.44	0.0008	2.14 serine palmitoyltransferase, long chain base subunit 1
VPS26	AF054179	12.00	2.92	5.62	4.35	0.0042	2.14 vacuolar protein sorting 26 (yeast homolog)
LMO4	U24576	33.20	9.28	15.54	10.52	0.0079	2.14 LIM domain only 4
TSC2	X75621	18.40	5.27	8.62	7.29	0.0101	2.14 tuberous sclerosis 2
GPS2	U28963	29.40	7.80	13.77	10.07	0.0082	2.14 G protein pathway suppressor 2
LOC51172	AF052111	40.40	11.44	18.92	11.54	0.0085	2.13 N-acetylglucosamine-1-phosphodiester alpha-N-acetylglucosaminidase
DKFZP566J15	AI857873	26.60	7.50	12.46	10.32	0.0092	2.13 DKFZP566J153 protein
SFRS5	U30826	26.60	5.81	12.46	8.47	0.0020	2.13 splicing factor, arginine/serine-rich 5
FIP2	AF070533	109.60	26.59	51.38	36.86	0.0039	2.13 tumor necrosis factor alpha-inducible cellular protein containing leucine zipper domains; Huntingtin interacting protein L; transcription factor IIIA-interacting prot
EEF1D	Z21507	26.40	4.39	12.38	7.79	0.0003	2.13 eukaryotic translation elongation factor 1 delta (guanine nucleotide exchange protein)
UNK_W27675	W27675	10.00	2.55	4.69	2.46	0.0051	2.13 Cluster Incl W27675: 36b3 Human retina cDNA randomly primed sublibrary Homo sapiens cDNA, mRNA sequence.
LOC55977	AI651358	31.80	5.31	14.92	7.24	0.0003	2.13 hypothetical protein 24636
UNK_AL04930	AL049309	35.40	6.91	16.62	12.09	0.0012	2.13 Homo sapiens mRNA; cDNA DKFZp564B176 (from clone DKFZp564B176)
PPP4C	X70218	37.20	9.63	17.46	12.31	0.0054	2.13 protein phosphatase 4 (formerly X), catalytic subunit
SAS10	AI128004	13.60	2.79	6.38	4.57	0.0016	2.13 disrupter of silencing 10
CD6	X60992	15.40	3.91	7.23	5.64	0.0054	2.13 CD6 antigen
KIAA0100	D43947	55.20	9.68	25.92	20.75	0.0011	2.13 KIAA0100 gene product
RBMS1	X77494	224.20	54.21	105.38	65.37	0.0038	2.13 RNA binding motif, single stranded interacting protein 1
UNK_W72239	W72239	10.80	2.28	5.08	3.28	0.0016	2.13 Homo sapiens mRNA; cDNA DKFZp434M162 (from clone DKFZp434M162)
KIAA1116	AB029019	19.80	5.36	9.31	6.02	0.0088	2.13 KIAA1116 protein
OXA1L	X80695	40.40	8.68	19.00	13.13	0.0019	2.13 oxidase (cytochrome c) assembly 1-like
ASMTL	AA889799	19.60	5.41	9.23	7.07	0.0081	2.12 acetylserotonin O-methyltransferase-like
MDS019	AL078641	22.20	4.60	10.46	7.42	0.0017	2.12 phorbolin-like protein MDS019
CREME9	AF046059	12.40	2.70	5.85	4.74	0.0028	2.12 cytokine receptor-like molecule 8
DAP3	U18321	12.40	3.29	5.85	5.37	0.0085	2.12 death associated protein 3
NME3	U29656	11.40	3.21	5.38	3.43	0.0085	2.12 non-metastatic cells 3, protein expressed in
CXAP1	D49738	32.40	4.04	15.31	10.19	0.0009	2.12 cytoskeleton-associated protein 1
ITGAM	J03925	14.00	2.45	6.62	4.39	0.0079	2.12 integrin, alpha M (complement component receptor 3, alpha; also known as CD11b (p170), macrophage antigen alpha polypeptide)
OAS2	M87434	16.60	3.65	7.85	5.68	0.0024	2.12 2-5oligoadenylate synthetase 2
BRF2	U07602	83.00	18.80	39.23	22.22	0.0025	2.12 butyrate response factor 2 (EGF-response factor 2)
USA-CYP	AF016371	117.00	31.44	55.31	37.35	0.0068	2.12 cyclophilin
KIAA0831	AB020638	46.20	8.35	21.85	11.88	0.0005	2.11 KIAA0831 protein
JAK1	M64174	14.80	2.49	7.00	4.12	0.0003	2.11 Janus kinase 1 (a protein tyrosine kinase)
LOC51614	AF091085	14.80	1.92	7.00	5.39	0.0003	2.11 hypothetical 43.2 Kd protein
UNK_AI890902	AI890903	30.40	7.16	14.38	12.80	0.0051	2.11 Cluster Incl AI890903: wm91f10.x1 NCI_CGAP_U2 Homo sapiens cDNA clone IMAGE:2443339 3'; mRNA sequence.
SMARCE1	AF035262	53.80	7.01	25.46	19.76	0.0003	2.11 SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily e, member 1
MLN64	D38255	73.60	19.48	34.85	21.49	0.0082	2.11 steroidogenic acute regulatory protein related
KIAA0579	AB011151	92.60	6.69	43.65	40.19	0.0009	2.11 KIAA0579 protein
UNK_AL04581	AL045811	34.80	0.56	16.62	11.00	0.0079	2.11 Homo sapiens mRNA; cDNA DKFZp434C0814 (from clone DKFZp434C0814)
RNAHP	AF083255	30.20	-4.82	14.31	9.61	0.0004	2.11 RNA helicase-related protein
SNRPN	U41303	11.20	3.19	5.31	3.12	0.0093	2.11 small nuclear ribonucleoprotein polypeptide N
KIAA0116	D29958	33.40	5.18	15.85	8.84	0.0002	2.11 KIAA0116 protein
KIAA0663	AB014563	15.40	2.70	7.31	4.92	0.0006	2.11 KIAA0663 gene product
TP53	M22898	15.40	4.04	7.31	5.31	0.0063	2.11 tumor protein p53 (Li-Fraumeni syndrome)

Table 7

Symbol	Accession						Description
BCS1L	AF038195	24.80	3.70	11.77	5.18	0.0001	2.11 BCS1 (yeast homolog)-like
PSMD1	D44466	50.40	11.33	23.92	15.96	0.0026	2.11 proteasome (prosome, macropain) 26S subunit, non-ATPase, 1
OXCT	U62961	16.20	4.15	7.69	5.47	0.0055	2.11 3-oxoacid CoA transferase
DKFZP547E21	AL050261	30.60	6.80	14.54	10.28	0.0026	2.10 DKFZP547E21D protein
LYPLA1	AF091281	20.40	5.16	9.69	6.07	0.0031	2.10 lysophospholipase I
UNK_L40402	L40402	17.00	3.32	8.08	6.66	0.0022	2.10 Homo sapiens mRNA; cDNA DKFZp434D0935 (from clone DKFZp434D0935)
KIAA1049	AB028972	17.80	2.28	8.46	5.83	0.0002	2.10 KIAA1049 protein
ABLIM	D31883	205.80	34.78	97.85	63.53	0.0005	2.10 actin binding LIM protein 1
HSF2	M65217	11.80	2.39	5.62	3.36	0.0022	2.10 heat shock transcription factor 2
RELA	L19067	11.80	2.39	5.62	3.91	0.0015	2.10 v-rel avian reticuloendotheliosis viral oncogene homolog A (nuclear factor of kappa light polypeptide gene enhancer in B-cells 3 (p65))
NFKB1	M58603	25.20	4.09	12.00	8.02	0.0004	2.10 nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)
UNK_U50535	U50535	38.60	6.54	18.38	10.87	0.0004	2.10 Human BRCA2 region, mRNA sequence CG006
SULT1A1	X78283	82.20	11.30	39.15	28.38	0.0003	2.10 sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1
DKFZP564O09	W21827	26.00	6.44	12.38	10.17	0.0057	2.10 DKFZP564O092 protein
DKFZP547E10	AF050260	21.80	5.76	10.38	9.40	0.0088	2.10 DKFZP547E1010 protein
UNK_2B2206	2B2206	17.60	1.95	8.38	5.72	0.0001	2.10 Cluster Incl 282206: Human DNA sequence from clone RP3-370A22 on chromosome 22 Contains part of the gene for ubiquinol-cytochrome c reductase iron-s
CLN3	U32680	26.80	3.63	12.77	8.83	0.0002	2.10 ceroid-lipofuscinosis, neuronal 3, juvenile (Batten, Spielmeyer-Vogt disease)
KIAA0618	AL080109	13.40	2.70	6.38	4.05	0.0013	2.10 KIAA0618 gene product
CO3D	AA919102	64.40	12.64	30.69	20.22	0.0012	2.10 CD3D antigen, delta polypeptide (TiT3 complex)
PCOLN3	U58048	10.00	2.55	4.77	4.40	0.0081	2.10 procollagen (type III) N-endopeptidase
UNK_AJ13311	AJ133115	36.60	9.74	17.46	13.20	0.0072	2.10 Homo sapiens mRNA for TSC-22-like protein
DKFZP727M23	AF055022	11.60	2.61	5.54	4.05	0.0030	2.09 DKFZP727M231 protein
WEE1	W28575	40.60	10.04	19.38	13.52	0.0047	2.09 wee1+ (S. pombe) homolog
FLJ20500	AA522530	17.40	3.91	8.31	5.60	0.0027	2.09 hypothetical protein
CD14	X06882	29.80	6.65	14.23	9.02	0.0025	2.09 CD14 antigen
GOLGA1	U51587	18.20	1.92	8.69	5.48	0.0011	2.09 golgi autoantigen, golgin subfamily a, 1
TBM44	AF026030	30.60	5.94	14.62	9.61	0.0011	2.09 translocase of inner mitochondrial membrane 44 (yeast) homolog
UNK_W26851	W26851	12.40	3.05	5.92	1.89	0.0061	2.09 ESTs, Weakly similar to ALU7_HUMAN ALU SUBFAMILY SQ SEQUENCE CONTAMINATION WARNING ENTRY [H.sapiens]
VPS45B	AJ133421	13.20	3.27	6.31	3.90	0.0046	2.09 vacuolar protein sorting 45B (yeast homolog)
PRKCB1	X07109	40.40	5.55	19.31	12.29	0.0002	2.09 protein kinase C, beta 1
DDX3	U50553	167.20	28.74	79.92	54.70	0.0006	2.09 DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 3
HSA9761	W67658	14.80	3.42	7.08	4.80	0.0032	2.09 putative dimethyladenosine transferase
MATK	S75168	14.80	3.35	7.08	4.91	0.0029	2.09 megakaryocyte-associated tyrosine kinase
KIAA0275	D87465	15.60	2.88	7.46	5.14	0.0009	2.09 KIAA0275 gene product
KIAA0438	AB007898	15.60	3.21	7.48	5.08	0.0017	2.09 KIAA0438 gene product
PRNP	U29185	23.80	2.17	11.38	6.13	0.0000	2.09 prion protein (p27-30) (Creutzfeld-Jakob disease, Gerstmann-Straussler-Scheinker syndrome, fatal familial insomnia)
HDAC6	AJ011972	47.40	8.38	22.69	17.73	0.0012	2.09 histone deacetylase 6
GRN	AF055008	21.20	5.89	10.15	7.12	0.0087	2.09 granulin
ITK	L10717	21.20	3.96	10.15	6.58	0.0009	2.09 IL2-inducible T-cell kinase
KIAA0244	D87685	44.80	7.19	21.46	14.91	0.0005	2.09 KIAA0244 protein
UBE2N	D83004	11.40	1.34	5.46	3.89	0.0002	2.09 ubiquitin-conjugating enzyme E2N (homologous to yeast UBC13)
HAX1	U68566	17.00	3.00	8.15	5.49	0.0007	2.08 HS1 binding protein
FCGR2A	M31932	37.20	6.72	17.85	13.27	0.0011	2.08 Fc fragment of IgG, low affinity IIa, receptor for (CD32)
SP2	D28588	18.60	2.07	8.92	6.47	0.0002	2.08 Sp2 transcription factor
CREB1	M27691	103.00	17.76	49.46	28.88	0.0005	2.08 cAMP responsive element binding protein 1
ABR	U01147	30.40	7.89	14.62	10.53	0.0064	2.08 active BCR-related gene
AMPEP	M22324	23.20	4.27	11.15	8.13	0.0011	2.08 alanyl (membrane) aminopeptidase (aminopeptidase N, aminopeptidase M, microsomal aminopeptidase, CD13, p150)
GABBR1	AJ225028	10.40	2.30	5.00	3.08	0.0025	2.08 gamma-aminobutyric acid (GABA) B receptor, 1
KIAA0179	D80001	20.00	4.74	9.62	6.96	0.0041	2.08 KIAA0179 protein
KIAA0630	AB014530	40.60	7.16	19.62	14.74	0.0010	2.08 KIAA0630 protein
SMAP	AW020536	20.60	2.68	10.00	5.42	0.0001	2.08 thyroid hormone receptor coactivating protein
UNK_AL03128	AL03128	54.40	8.53	26.15	17.09	0.0004	2.08 Cluster Incl AL031282: Human DNA sequence from clone 283E3 on chromosome 1p36.21-35.33. Contains the alternatively spliced gene for Matrix Metallic
EED	AF080227	68.20	11.43	32.85	30.82	0.0027	2.08 embryonic ectoderm development
ARHGEF1	Y09160	40.40	3.97	19.46	13.69	0.0001	2.08 Rho guanine nucleotide exchange factor (GEF) 1
PIP5K2B	U85245	18.20	4.76	8.77	6.86	0.0073	2.08 phosphatidylinositol-4-phosphate 5-kinase, type II, beta
UNK_N36842	N36842	18.20	4.68	8.77	6.31	0.0061	2.08 Homo sapiens cDNA FLJ10207 fis, clone HEMBA1005475
SDHA	L21936	17.40	3.21	8.38	5.58	0.0009	2.08 succinate dehydrogenase complex, subunit A, flavoprotein (Fp)
BTG2	U72649	13.40	3.05	6.46	4.16	0.0030	2.07 BTG family, member 2
RPC	Y11651	13.40	3.65	6.46	3.97	0.0079	2.07 RNA 3'-terminal phosphate cyclase
KIAA0135	D50925	64.60	8.65	31.15	22.74	0.0004	2.07 KIAA0135 protein
KIAA0257	D87446	12.60	1.52	6.08	3.30	0.0000	2.07 KIAA0257 protein
UNK_AF00978	AF009787	12.60	2.70	6.08	5.20	0.0038	2.07 Homo sapiens T cell receptor beta chain (TCRB) mRNA, partial cds
HLA-G	M90683	35.20	8.47	17.46	11.84	0.0038	2.07 HLA-G histocompatibility antigen, class I, G
STX7	U77942	11.80	2.49	5.69	3.54	0.0019	2.07 syntaxin 7
MAEA	AF084928	11.00	2.35	5.31	4.35	0.0032	2.07 macrophage erythroblast attacher
SFRS11	N74002	11.00	2.45	5.31	2.36	0.0029	2.07 splicing factor, arginine/serine-rich 11
ARL7	AB016811	10.20	0.84	4.92	3.62	0.0002	2.07 ADP-ribosylation factor-like 7
IGHD	X02882	10.20	2.39	4.92	3.62	0.0040	2.07 immunoglobulin heavy constant delta
TPM4	X05276	10.20	2.39	4.92	3.28	0.0036	2.07 tropomyosin 4

Table 7

Gene	Accession					p-value	Ratio	Description
UNK_AI01809E	AI018098	256.20	51.21	123.69	70.16	0.0013	2.07	Cluster Incl AI018098: ov65b11.s1 Soares_testis_NHT Homo sapiens cDNA clone IMAGE:1642173 3', mRNA sequence.
GG2-1	AF099935	17.20	4.71	8.31	6.21	0.0088	2.07	TNF-induced protein
RAC2	M64595	68.80	11.95	33.23	17.07	0.0005	2.07	ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2)
SLC2A3	M20681	26.40	4.16	12.77	8.34	0.0004	2.07	solute carrier family 2 (facilitated glucose transporter), member 3
BTN2A1	U90543	19.40	2.07	9.38	6.92	0.0003	2.07	butyrophilin, subfamily 2, member A1
CCT2	AF026166	28.60	5.90	13.85	9.79	0.0020	2.07	chaperonin containing TCP1, subunit 2 (beta)
GLUL	X59834	28.60	7.86	13.85	5.93	0.0094	2.07	glutamate-ammonia ligase (glutamine synthase)
PNN	U77718	17.00	2.92	8.23	5.43	0.0006	2.07	pinin, desmosome associated protein
FASTK	X86779	10.80	1.48	5.23	3.42	0.0002	2.06	Fas-activated serine/threonine kinase
HSGT1	D88208	10.80	2.39	5.23	5.07	0.0068	2.06	suppressor of S. cerevisiae gcr2
LYN	M79321	64.00	14.87	31.00	22.82	0.0040	2.06	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog
ZNF278	AL096890	10.00	1.58	4.85	2.41	0.0002	2.06	zinc finger protein 278
KIAA0370	AB002368	14.60	2.88	7.08	4.42	0.0013	2.06	KIAA0370 protein
NOT56L	Y09022	14.80	2.30	7.08	5.06	0.0006	2.06	Nol5b (D. melanogaster)-like protein
UNK_W28732	W28732	115.20	18.19	55.85	26.05	0.0002	2.06	Cluster Incl W28732: 50h7 Human retina cDNA randomly primed sublibrary Homo sapiens cDNA, mRNA sequence.
KIAA1046	AB028969	13.00	2.24	6.31	4.23	0.0007	2.06	KIAA1046 protein
C22ORF4	AL096779	109.80	24.03	53.31	36.41	0.0032	2.06	chromosome 22 open reading frame 4
UNK_AL04941	AL049415	12.20	1.92	5.92	3.75	0.0004	2.06	Homo sapiens mRNA; cDNA DKFZp586N2119 (from clone DKFZp586N2119)
PIG7	AL120815	32.00	5.24	15.54	11.46	0.0008	2.06	LPS-induced TNF-alpha factor
XPOT	AF039022	18.20	2.28	8.85	4.08	0.0000	2.06	exportin, tRNA (nuclear export receptor for tRNAs)
KIAA0978	AB023195	69.60	14.76	33.85	18.58	0.0020	2.06	KIAA0978 protein
UNK_AL02239	AL022398	112.40	25.21	54.89	31.85	0.0028	2.06	Cluster Incl AL022398: Homo sapiens DNA sequence from PAC 434O14 on chromosome 1q32.3.-41. Contains the HSD11B1 gene for Hydroxysteroid (11-beta
37137	299716	41.40	6.88	20.15	13.50	0.0006	2.05	septin 3
CDBA	M12824	15.00	3.87	7.31	4.82	0.0049	2.05	CD8 antigen, alpha polypeptide (p32)
KIAA0126	D50918	33.00	5.70	16.08	12.03	0.0011	2.05	KIAA0126 protein; septin 2
LOC64116	AL049963	15.00	4.06	7.31	4.57	0.0081	2.05	up-regulated by BCG-CWS
NFYA	AL031778	54.00	15.22	26.31	16.11	0.0099	2.05	nuclear transcription factor Y, alpha
FLJ20552	AI827877	20.20	5.02	9.85	8.00	0.0067	2.05	hypothetical protein FLJ20552
UNK_AL08011	AL080111	14.20	3.70	6.92	3.20	0.0071	2.05	Homo sapiens mRNA; cDNA DKFZp586G2222 (from clone DKFZp586G2222)
CCM1	U90268	11.20	2.95	5.46	3.76	0.0073	2.05	cerebral cavernous malformations 1
IFNGR2	U05875	43.20	8.98	21.08	14.59	0.0021	2.05	interferon gamma receptor 2 (interferon gamma transducer 1)
SNW1	AF045184	21.60	3.21	10.54	7.14	0.0004	2.05	SKI-INTERACTING PROTEIN
CHRNB1	X14830	10.40	2.61	5.08	3.23	0.0055	2.05	cholinergic receptor, nicotinic, beta polypeptide 1 (muscle)
MAPK9	U09759	10.40	2.79	5.08	3.66	0.0003	2.05	mitogen-activated protein kinase 9
DIA1	M28713	57.60	8.56	28.15	17.47	0.0003	2.05	diaphorase (NADH) (cytochrome b-5 reductase)
ZNFN1A1	U40462	148.40	8.20	72.54	50.50	0.0001	2.05	zinc finger protein, subfamily 1A, 1 (Ikaros)
LMNB2	M94362	50.20	9.63	24.54	17.46	0.0015	2.05	lamin B2
RASGRP1	AF081195	30.20	8.04	14.77	7.79	0.0077	2.04	RAS guanyl releasing protein 1 (calcium and DAG-regulated)
CNP	D13146	18.40	2.97	9.00	4.74	0.0003	2.04	2',3'-cyclic nucleotide 3' phosphodiesterase
KIAA0870	AB020677	82.40	17.07	40.31	29.60	0.0024	2.04	Cluster Incl AB020677
MAAT1	U19796	31.60	4.16	15.46	10.92	0.0003	2.04	melanoma-associated antigen recognised by cytotoxic T lymphocytes
CACT	Y10319	41.80	7.48	20.46	12.17	0.0007	2.04	carnitine/acylcarnitine translocase
KIAA0253	D87442	13.20	3.56	6.46	4.29	0.0053	2.04	nicastrin
SDHD	AB006202	13.20	3.35	6.46	4.26	0.0060	2.04	succinate dehydrogenase complex, subunit D, integral membrane protein
TASR1	AF047448	11.00	2.45	5.38	3.25	0.0028	2.04	TLS-associated serine-arginine protein 1
UNK_N30151	N30151	11.00	1.41	5.38	3.91	0.0004	2.04	Cluster Incl N30151: yx81f01.s1 Soares melanocyte 2NbHM Homo sapiens cDNA clone IMAGE:268153 3'. mRNA sequence.
SFPQ	W27611	38.80	9.20	19.00	12.86	0.0043	2.04	splicing factor proline/glutamine rich (polypyrimidine tract-binding protein-associated)
USP24	AB028980	32.20	7.92	15.77	10.12	0.0051	2.04	ubiquitin specific protease 24
AP3M2	D38293	27.80	3.03	13.62	7.31	0.0000	2.04	adaptor-related protein complex 3, mu 2 subunit
FOS	V01512	27.80	7.73	13.62	9.83	0.0100	2.04	v-fos FBJ murine osteosarcoma viral oncogene homolog
EBI2	L08177	21.20	4.02	10.38	8.45	0.0011	2.04	Epstein-Barr virus induced gene 2 (lymphocyte-specific G protein-coupled receptor)
HSU15552	U15552	14.60	4.04	7.15	4.08	0.0093	2.04	acidic 82 kDa protein mRNA
MYPT1	D87930	14.60	2.70	7.15	5.13	0.0014	2.04	myosin phosphatase, target subunit 1
NCK2	AI275502	14.60	2.07	7.15	4.49	0.0002	2.04	NCK adaptor protein 2
SYNE-1B	AB018339	14.60	3.51	7.15	4.72	0.0048	2.04	synaptic nuclei expressed gene 1b
UNK_AF05335	AF053356	14.60	3.13	7.15	5.89	0.0039	2.04	Cluster Incl AF053356: Homo sapiens chromosome 7q22 sequence, complete sequence.
RMP	AB006572	19.60	1.52	9.62	6.24	0.0001	2.04	RPB5-mediating protein
EIF1A	L18960	15.20	4.15	7.48	5.53	0.0095	2.04	eukaryotic translation initiation factor 1A
ELF4	U32645	18.80	4.97	9.23	6.50	0.0079	2.04	E74-like factor 4 (ets domain transcription factor)
KIAA0397	AB007857	15.80	3.35	7.77	5.56	0.0027	2.03	KIAA0397 gene product
RNASE2	X55988	12.20	3.03	6.00	4.51	0.0063	2.03	ribonuclease, RNase A family, 2 (liver, eosinophil-derived neurotoxin)
NDUFS8	AF038406	41.60	8.44	20.46	13.56	0.0019	2.03	NADH dehydrogenase (ubiquinone) Fe-S protein 8 (23kD) (NADH-coenzyme Q reductase)
DKF2P434M18	AL080141	18.60	4.04	9.15	5.21	0.0025	2.03	DKF2P434M183 protein
76P	AA058852	10.00	2.65	4.92	3.50	0.0081	2.03	gamma tubulin ring complex protein (76p gene)
ARS2	AL096723	20.00	4.74	9.85	4.72	0.0044	2.03	arsenate resistance protein ARS2
C2F	U72514	55.00	11.25	27.08	17.99	0.0020	2.03	putative protein
KIAA0456	AB007925	10.00	2.00	4.92	2.60	0.0015	2.03	KIAA0456 protein
SLU7	AI860856	26.40	8.02	13.00	10.41	0.0049	2.03	step II splicing factor SLU7
HNRPC	M16342	11.40	2.70	5.62	3.15	0.0042	2.03	heterogeneous nuclear ribonucleoprotein C (C1/C2)

Table 7

Symbol	Accession							Description
PRPS1	D00860	11.40	2.88	5.62	3.75	0.0062	2.03	phosphoribosyl pyrophosphate synthetase 1
IL11RA	U32324	24.20	3.83	11.92	6.42	0.0005	2.03	Interleukin 11 receptor, alpha
KIAA0276	D87468	12.80	1.48	6.31	3.88	0.0001	2.03	KIAA0276 protein
HNRPD	M94630	49.00	8.37	24.15	14.56	0.0006	2.03	heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA-binding protein 1, 37kD)
RAB1	AL050268	19.80	3.70	9.77	7.49	0.0019	2.03	RAB1, member RAS oncogene family
D1042K10.2	AL022238	10.60	2.07	5.23	2.86	0.0013	2.03	hypothetical protein
DDOST	D29643	24.00	6.24	11.85	8.49	0.0076	2.03	dolichyl-diphosphooligosaccharide-protein glycosyltransferase
ERH	D85758	24.00	6.20	11.85	7.34	0.0068	2.03	enhancer of rudimentary (Drosophila) homolog
RPS6KA3	U08316	167.20	31.60	82.54	48.48	0.0011	2.03	ribosomal protein S6 kinase, 90kD, polypeptide 3
DKFZP586O16	AL039458	13.40	3.36	6.62	5.47	0.0079	2.03	DKFZP586O1624 protein
NTE	AJ004832	40.80	4.44	20.15	10.48	0.0000	2.02	neuropathy target esterase
MGEA5	AB014579	26.00	5.10	12.85	7.17	0.0013	2.02	meningioma expressed antigen 5 (hyaluronidase)
UNK_AA17678	AA176780	81.60	15.93	30.46	16.03	0.0069	2.02	Cluster Incl AA176780: zp52a10.s1 Stratagene neuroepithelium (#937231) Homo sapiens cDNA clone IMAGE:611130 3' similar to contains Alu repetitive elem
RERE	AB007927	14.00	3.24	6.92	4.82	0.0042	2.02	arginine-glutamic acid dipeptide (RE) repeats
KIAA1115	AB029038	37.00	8.96	18.31	12.91	0.0016	2.02	KIAA1115 protein
KIAA0154	D63876	163.20	40.83	80.77	56.67	0.0063	2.02	KIAA0154 protein; ADP-ribosylation factor binding protein GGA3
IFI6	M63338	16.00	3.32	7.92	4.68	0.0020	2.02	Interferon, gamma-inducible protein 16
RNF15	U90547	16.00	2.45	7.92	5.74	0.0007	2.02	ring finger protein 15
MOF	AL050395	43.80	11.17	21.69	14.66	0.0067	2.02	member of MYST family histone acetyl transferases, homolog of Drosophila MOF
DSIPI	Z50781	55.60	13.59	27.54	20.04	0.0059	2.02	delta sleep inducing peptide, immunoreactor
KIAA0981	AB023198	11.80	2.77	5.85	5.26	0.0078	2.02	KIAA0981 protein
FLJ20297	AF052134	19.40	4.28	9.62	8.11	0.0052	2.02	hypothetical protein FLJ20297
ADAM10	Z48579	15.20	3.27	7.54	5.90	0.0038	2.02	a disintegrin and metalloprotease domain 10
KIAA0543	AB011115	30.40	7.85	15.08	9.54	0.0071	2.02	KIAA0543 protein
VEGFB	U43368	22.80	2.95	11.31	7.30	0.0002	2.02	vascular endothelial growth factor B
KIAA0747	AB018290	56.60	14.79	28.08	23.28	0.0097	2.02	KIAA0747 protein
UNK_ALD4759	ALD47596	41.40	7.64	20.54	11.84	0.0009	2.02	Homo sapiens cDNA: FLJ21480 fis, clone COL05034
UNK_AL05037	AL050376	33.80	5.89	16.77	11.40	0.0010	2.02	Homo sapiens mRNA; cDNA DKFZp586J101 (from clone DKFZp586J101)
BIN1	AF001383	29.60	7.09	14.69	9.96	0.0050	2.01	bridging integrator 1
CSPG6	AF020043	23.40	5.59	11.62	8.26	0.0052	2.01	chondroitin sulfate proteoglycan 6 (bamacan)
H326	U06631	17.20	3.70	8.54	5.25	0.0026	2.01	H326
UP	X9055	34.40	7.63	17.08	12.64	0.0044	2.01	uridine phosphorylase
TPST2	AF049891	111.20	13.41	55.23	32.14	0.0001	2.01	tyrosylprotein sulfotransferase 2
CL24751	AF070530	17.80	2.17	8.85	4.62	0.0001	2.01	hypothetical protein, clone 24751
APB81	L77864	13.00	3.16	6.46	4.41	0.0055	2.01	amyloid beta (A4) precursor protein-binding, family B, member 1 (Fe65)
KHSRP	A626946	13.00	2.12	6.46	4.88	0.0012	2.01	KH-type splicing regulatory protein (FUSE binding protein 2)
COPS3	AF031647	21.20	3.96	10.54	7.09	0.0014	2.01	COP9 (constitutive photomorphogenic, Arabidopsis, homolog) subunit 3
ROCK1	U43195	24.60	3.85	12.23	7.79	0.0005	2.01	Rho-associated, coiled-coil containing protein kinase 1
BAP1	AF045581	16.40	4.28	8.15	4.43	0.0074	2.01	BRCA1 associated protein-1 (ubiquitin carboxy-terminal hydrolase)
CRBP	D78134	16.40	4.16	8.15	5.65	0.0069	2.01	cold inducible RNA-binding protein
DKFZP586O02	AL098741	23.20	5.93	11.54	8.54	0.0077	2.01	hypothetical protein
PAPSS1	Y10087	40.20	9.20	20.00	10.89	0.0035	2.01	3-prime-phosphoadenosine 5-prime-phosphosulfate synthase 1
MAF	AF055376	66.60	14.62	33.15	23.10	0.0034	2.01	v-maf musculoaponeurotic fibrosarcoma (avian) oncogene homolog
HLA-DQB1	M16276	48.20	8.53	24.00	20.43	0.0028	2.01	major histocompatibility complex, class II, DQ beta 1
ITPR3	U01062	12.20	1.48	6.08	3.25	0.0001	2.01	inositol 1,4,5-triphosphate receptor, type 3
DYRK1A	D86550	58.20	13.33	29.00	17.54	0.0037	2.01	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A
CD3Z	J04132	31.80	7.82	15.85	10.00	0.0056	2.01	CD3Z antigen, zeta polypeptide (TiT3 complex)
AES	AI951946	23.00	3.46	11.46	4.99	0.0092	2.01	amino-terminal enhancer of split
PTPN1	M31724	28.40	6.35	14.15	9.66	0.0002	2.01	protein tyrosine phosphatase, non-receptor type 1
AMD1	M21154	19.60	4.04	9.77	5.69	0.0037	2.01	S-adenosylmethionine decarboxylase 1
KIAA0810	AB018353	68.20	12.56	33.00	19.00	0.0020	2.01	KIAA0810 protein
KIAA0107	D14663	25.60	6.47	12.77	9.02	0.0012	2.00	KIAA0107 gene product
PRSS15	X76040	155.60	16.32	77.62	48.66	0.0001	2.00	protease, serine, 15
NR3C1	M10901	200.60	28.11	100.08	61.50	0.0003	2.00	nuclear receptor subfamily 3, group C, member 1
RNF6	AJ010346	33.80	5.68	16.77	10.05	0.0006	2.00	ring finger protein (C3HC3 type)
ISG20	U88964	28.20	7.46	14.08	10.44	0.0092	2.00	interferon stimulated gene (20kD)
UNK_U23852	U23852	15.40	2.61	7.69	4.75	0.0007	2.00	Cluster Incl U23852: Human T-lymphocyte specific protein tyrosine kinase p56lck (lck) aberrant mRNA, complete cds.
CBFB	L20298	17.40	4.34	8.69	7.30	0.0087	2.00	core-binding factor, beta subunit
ATP5J	AA845575	31.40	7.57	15.69	11.33	0.0056	2.00	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit F6
UNK_AF02326	AF023268	33.40	6.23	16.69	12.11	0.0073	2.00	Homo sapiens cDNA: FLJ23280 fis, clone COL05804, highly similar to HSU90911 Human clone 23652 mRNA sequence
UNK_U90911	U90911	35.40	8.10	17.69	11.65	0.0074	2.00	unchancterized bone marrow protein BM036
BM036	AI057607	16.00	4.08	-8.00	5.70	0.0041	2.00	
JMJ	AL021936	16.00	3.67	8.00	5.23	0.0045	2.00	jumonji (mouse) homolog
USPI9X	X98266	18.00	2.12	9.00	9.06		2.00	ubiquitin specific protease 9, X chromosome (Drosophila fat facets related)

Table 8

Gene Name	Accession #	p value	ave nom/unlnv	sd nom/unlnv	ave chronic	sd chronic	fc chron/unlnv	gene description
CCT3	X74801	0.009937	2.75	1.83	35.20	27.31	12.80	CCT3
GRHPR	W29944	0.009949	2.35	1.06	18.40	15.22	7.75	glyoxylate reductase/hydroxypyruvate reductase
ZNF212	U38864	0.008821	3.38	0.92	12.60	6.31	3.73	zinc finger protein 212
RPS3	X55715	0.010192	2.88	2.64	10.60	1.67	3.69	RPS3
SPARC	J03040	0.009001	4.13	4.52	10.00	2.55	2.42	secreted protein, acidic, cysteine-rich (osteonectin)
HLA-DPB1	M83664	0.009253	6.25	3.92	14.20	5.81	2.27	major histocompatibility complex, class II, DP beta 1
RAN	M31469	0.009278	9.50	5.15	21.40	7.89	2.25	RAN, member RAS oncogene family
GSN	X04412	0.00932	6.13	2.59	13.60	5.37	2.22	GSN
KIAA0992	AB023209	0.009415	4.63	1.51	10.20	6.69	2.21	KIAA0992 protein
SEC14L1	D67029	0.009467	12.88	5.72	28.20	10.69	2.19	SEC14 (S. cerevisiae)-like 1
PLTP	L26232	0.009516	6.88	4.36	15.00	5.66	2.18	phospholipid transfer protein
VIM	Z19554	0.009943	19.50	8.50	41.20	9.83	2.11	VIM
RARRES3	AF060228	0.009658	22.75	9.27	47.20	19.46	2.07	retinoic acid receptor responder (tazarotene induced) 3
UNK_L27065	L27065	0.00848	6.00	5.07	12.40	6.58	2.07	Neurofibromatosis 2 Tumor Suppressor
SAH	X00062	0.009295	16.00	8.49	8.00	1.22	0.50	SAH
MADH5	AF010607	0.001577	28.00	15.10	14.00	2.00	0.50	MAD (mothers against decapentaplegic, Drosophila) homolog 5
IGHMBP2	L14754	0.004237	19.63	9.13	9.80	2.17	0.50	Immunoglobulin mu binding protein 2
XBP1	Z93930	0.004382	25.25	13.09	12.60	4.04	0.50	X-box binding protein 1
CALCR	L00587	0.004237	51.75	25.04	25.80	8.58	0.50	calcitonin receptor
MDFI	U78313	0.00308	62.63	30.50	31.00	9.92	0.50	MyoD family inhibitor
UNK_AL050030	AL050030	0.003109	13.38	5.18	6.50	2.19	0.49	UNK_AL050030
UNK_W26326	W26326	0.001916	53.86	24.79	26.20	11.03	0.49	Homo sapiens DNA, cosmid clones TN62 and TN82
TUBA3	X01703	0.001219	12.50	5.63	6.00	1.22	0.48	TUBA3
SPUF	AA863101	0.007552	65.25	33.80	30.40	7.96	0.47	secreted protein of unknown function
NFE2L1	L24123	0.006546	26.38	13.29	13.20	2.05	0.47	NFE2L1
COL18A1	AF018081	0.004472	61.38	32.49	28.40	2.51	0.46	collagen, type XVIII, alpha 1
UNK_W27871	W27871	0.004279	42.63	20.56	19.60	2.70	0.46	Cluster Incl W27871: 35a1 Human retina cDNA randomly primed sublibrary Homo sapiens cDNA, mRNA sequence
HSPA2	L26336	0.004481	23.63	10.74	10.80	2.68	0.46	heat shock 70kD protein 2
FRAP1	X29031	0.00772	26.88	13.46	12.00	4.90	0.45	ESTs, Weakly similar to atrophin-1 related protein [H.sapiens]
UNK_AI290480	AI290480	0.007803	26.50	16.35	11.80	3.27	0.45	ESTs, Moderately similar to JC4969 pig-c protein - human [H.sapiens]
KIAA0477	AB007846	0.006066	88.50	47.91	39.20	14.41	0.44	KIAA0477 gene product
MED6	U78082	0.005343	13.13	5.77	5.80	1.79	0.44	RNA polymerase II transcriptional regulation mediator (Med6, S. cerevisiae, homolog of)
SMTN	AB88563	0.005403	24.00	14.08	10.60	3.36	0.44	Inositol polyphosphate 5-phosphatase
MLLT4	AB011399	0.004237	150.38	70.56	65.80	15.83	0.44	myeloid/lymphoid or mixed-lineage leukemia (trithorax (Drosophila) homolog); translocated to, 4
LLGL1	AW007029	0.004429	12.38	6.93	5.40	1.14	0.44	lethal giant larvae (Drosophila) homolog 1
UNK_W29012	W29012	0.004233	55.00	24.12	24.00	1.00	0.44	Cluster Incl W29012: 55a6 Human retina cDNA randomly primed sublibrary Homo sapiens cDNA, mRNA sequence
PIGR	X73079	0.003749	87.75	38.30	38.20	8.44	0.44	PIGR
TBL1	AA149637	0.005301	57.25	27.37	24.80	6.22	0.43	transducin (beta)-like 1
KIAA0210	D86965	0.004846	38.88	18.86	16.80	2.77	0.43	KIAA0210 gene product
LTK	D16105	0.004528	19.00	9.53	8.20	2.68	0.43	leukocyte tyrosine kinase
RPS17	M13932	0.004379	14.38	7.39	6.20	1.48	0.43	ribosomal protein S17
GRSF1	U07231	0.004362	28.88	13.57	12.40	2.07	0.43	G-rich RNA sequence binding factor 1
UNK_AL022398	AL022398	0.004054	20.50	8.70	8.80	3.66	0.43	Cluster Incl AL022398: Homo sapiens DNA sequence from PAC 434O14 on chromosome 1q32.3.-41.
GATA2	M77810	0.00394	17.75	10.46	7.60	2.51	0.43	GATA-binding protein 2
PABPC1	Y00345	0.003317	27.63	11.90	11.60	2.77	0.43	PABPC1
MPHOSPH9	N23137	0.002013	10.38	4.93	4.40	1.14	0.42	M-phase phosphoprotein 9
NFKB1	M55603	0.002132	10.88	5.28	4.60	1.14	0.42	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)
SNRPG	AB03447	0.00347	59.00	31.72	24.30	7.26	0.42	small nuclear ribonucleoprotein polypeptide G
KRTHA3B	X82634	0.001769	11.50	6.07	4.80	1.48	0.42	KRTHA3B
CLUL1	D63813	0.00245	21.63	10.97	9.00	2.45	0.42	clusterin-like 1 (retinal)
PMS2	U13696	0.001807	48.63	20.07	19.40	3.91	0.42	postmeiotic segregation increased (S. cerevisiae) 2
MGAT2	U15128	0.002266	12.13	6.42	5.00	1.22	0.41	mannosyl (alpha-1,6-)glycoprotein beta-1,2-N-acetylglucosaminyltransferase
UBE2L6	AA883502	0.003066	15.63	7.82	6.40	1.14	0.41	ubiquitin-conjugating enzyme E2L 6
GYPE	X53004	0.002639	16.13	9.67	6.60	2.07	0.41	GYPE
IRS1	S62539	0.001956	89.38	43.55	36.40	12.56	0.41	insulin receptor substrate 1
ITGB3	M25108	0.001833	10.50	6.78	4.20	0.84	0.40	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)
CDK5R1	X80343	0.004472	16.63	7.93	6.20	3.19	0.37	CDK5R1
UNK_AI557322	AI557322	0.004237	17.25	8.58	6.40	2.07	0.37	Cluster Incl AI557322: PT2.1_16_F11.r tumor2 Homo sapiens cDNA 3'; mRNA sequence.
LOC55977	AF070843	0.004237	32.33	17.14	12.00	1.73	0.37	LOC55977
HIPK3	AF004849	0.002643	11.38	6.48	4.20	1.30	0.37	homeodomain-interacting protein kinase 3
PTPN21	X79510	0.002454	17.88	10.92	6.60	1.14	0.37	PTPN21

Table 8

UNK_X89059	X89059	5.03E-05	21.13	10.70	7.40	1.14	0.35	STK9
KIAA0744	AB018287	0.00017	18.88	11.47	8.60	2.51	0.35	KIAA0744 gene product; histone deacetylase 7
SLC18A2	L14269	0.01401	23.88	16.39	8.20	1.92	0.34	solute carrier family 18 (vesicular monoamine), member 2
CELSR1	AI275081	0.000276	25.25	13.22	8.40	2.19	0.33	cadherin EGF LAG seven-pass G-type receptor
THPO	L33410	0.001154	46.25	23.27	15.00	3.00	0.32	thrombopoietin (myeloproliferative leukemia virus oncogene ligand, megakaryocyte growth and development factor
KIAA0388	AB002366	0.000906	10.50	8.14	3.40	0.55	0.32	KIAA0388 protein
UNK_W25917	W25917	0.001177	18.75	10.05	5.40	1.67	0.32	Cluster Incl W25917: 14n6 Human retina cDNA randomly primed sublibrary Homo sapiens cDNA, mRNA sequence
CXADR	Y07593	0.000704	10.63	8.86	3.40	1.14	0.32	CXADR
NME2	X58965	0.000814	54.00	28.17	16.40	2.88	0.30	NME2
ELK1	AB016194	0.000592	18.50	9.18	5.60	1.67	0.30	ELK1, member of ETS oncogene family
NACA	AF054187	0.000396	11.25	7.34	3.40	1.14	0.30	nascent-polypeptide-associated complex alpha polypeptide
ATP5G3	U09813	0.000457	21.25	11.36	6.40	1.52	0.30	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9) isoform 3
ATP7B	U11700	0.000559	14.75	8.38	4.40	0.89	0.30	ATPase, Cu++ transporting, beta polypeptide (Wilson disease)
HIPK3	AJ523538	0.00053	23.50	12.71	7.00	2.45	0.30	homeodomain-interacting protein kinase 3
FLJ10871	U96629	0.000317	12.13	6.56	3.60	0.89	0.30	UNK_U96629
TRHR	D85376	6.29E-05	12.88	6.94	3.80	1.30	0.30	thyrotropin-releasing hormone receptor
DRD3	U25441	9.9E-06	30.50	15.69	9.00	3.67	0.30	dopamine receptor D3
MYO9B	U42391	1.44E-05	10.88	7.28	3.20	1.64	0.29	myosin IXB
HYPB	AL049470	2.26E-05	12.25	6.69	3.60	1.67	0.29	HYPB
CASP8	X98176	0.000349	11.63	6.25	3.40	1.14	0.29	CASP8
RFPL3	AJ010232	6.98E-05	43.13	23.24	12.60	5.13	0.29	RFPL3
SCN1B	L10338	2.89E-05	16.63	9.53	4.80	2.39	0.29	sodium channel, voltage-gated, type I, beta polypeptide
ARC	D87468	3.48E-05	24.25	17.43	7.00	3.32	0.29	activity-regulated cytoskeleton-associated protein
EGR1	X52541	4.09E-05	10.50	6.52	3.00	0.00	0.29	early growth response 1
UNK_M21259	M21259	0.000202	16.25	8.83	4.60	1.52	0.28	small nuclear ribonucleoprotein polypeptide E
DSPG3	U59111	9.61E-05	17.00	9.50	4.80	0.84	0.28	dermatan sulphate proteoglycan 3
ITK	L10717	0.000154	10.13	7.95	2.80	1.30	0.28	IL2-inducible T-cell kinase
NHLH2	M98740	4.18E-05	10.13	5.69	2.80	1.30	0.28	NHLH2
SREC	D86864	9.83E-05	17.38	9.26	4.80	1.48	0.28	acetyl LDL receptor; SREC
ZNF148	AJ236885	0.00039	10.88	9.72	3.00	0.71	0.28	ZNF148
SMARCD1	U66617	0.000118	13.88	7.77	3.80	1.30	0.27	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 1
NN8-4AG	U50383	0.000194	10.63	6.44	2.60	0.55	0.24	retinoic acid responsive
UNK_M20642	M20642	0.000122	14.00	8.41	3.40	1.52	0.24	Human alkali myosin light chain 3 mRNA, complete cds
GFAP	S40719	0.000161	10.75	6.94	2.60	0.89	0.24	glial fibrillary acidic protein
KIAA0847	AB020654	0.000141	14.38	8.02	3.40	0.89	0.24	KIAA0847 protein

Table 9

Genes Differentially regulated in Murine EAE and Human MS

Gene Name	Murine Accession	Description
EEF1D	aa253918	Cluster Incl Z21507: H.sapiens EF-1delta gene encoding human elongation factor-1-delta.
PIM2	Msa.2067.0	Human pim-2 protooncogene homolog pim-2h mRNA, complete cds.
PRDX2	Z21848	Cluster Incl L19185: Human natural killer cell enhancing factor (NKEFB) mRNA, complete cds.
SEC24C	aa175517	Cluster Incl D38555: Human mRNA for KIAA0079 gene, complete cds.
UNK_AJ2454	u85207	Cluster Incl AJ245416: Homo sapiens mRNA for G7b protein (G7b gene, located in the class III region of the major histocompatibility complex.
XIP	Msa.3679.0	Cluster Incl AF029890: Homo sapiens hepatitis B virus X interacting protein (XIP) mRNA, complete cds.

Table 10

	A	B	C	D	E
1	Peripheral Blood Genes Predicitve of Multiple Sclerosis				
2					
3	Gene	Observed	Random Perm 1%	Random Perm 5%	Randon Perm (Median)
4	FOS	2.562527	1.4817816	1.1614933	0.7007793
5	U19261	2.114882	1.3043897	0.87986296	0.53156734
6	DUSP1	1.939225	1.2410891	0.8299547	0.5107011
7	KIAA0906	1.87203	1.2133218	0.80576986	0.48326102
8	MAP4K1	1.860437	1.2085855	0.79408497	0.47091565
9	DGKZ	1.837408	1.2069645	0.77625513	0.4690797
10	SNRP70	1.81934	1.1756094	0.7738778	0.45766842
11	ETR101	1.801398	1.1731951	0.76643366	0.440602
12	KIAA0864	1.775063	1.1709846	0.7597211	0.43995833
13	SFRS8	1.77253	1.1582581	0.7580478	0.43687758
14	AI890903	1.764722	1.1560028	0.7550534	0.4319513
15	JUNB	1.752881	1.1488212	0.74930257	0.4269654
16	STAT4	1.719926	1.1444192	0.7461597	0.41349095
17	AQP3	1.705897	1.130565	0.7446831	0.4113997
18	HSU79253	1.664187	1.1186187	0.7290762	0.4062087
19	BIN1	1.649725	1.1111517	0.7287055	0.402228
20	ATM	1.641806	1.1030456	0.7274922	0.39897352
21	EDG4	1.634613	1.0987875	0.7267664	0.39316127
22					
23	Prediction based on correlation metrics proposed by Gollub et. al. (1999)				

- 162 -

WHAT IS CLAIMED:

1.   A method of diagnosing a subject with multiple
sclerosis, the method comprising the step of comparing:

a) a level of expression of a marker in a sample
from the subject, wherein the marker is selected from
the group consisting of markers listed in Tables 7-10,
and

b) a normal level of expression of the marker in a
control sample,

wherein a substantial difference between the level
of expression of the marker in the sample from the
subject and the normal level is an indication that the
subject is afflicted with multiple sclerosis.

2.   The method of claim 1, wherein the marker
corresponds to a transcribed polynucleotide or a
portion thereof.

3.   The method of claim 2, wherein the sample is
collected from brain tissue.

4.   The method of claim 2, wherein the sample is
peripheral blood mononuclear cells.

5.   The method of claim 1, wherein the control sample
is from a non-diseased subject and the substantial
difference is a factor of at least about 2.

6.   The method of claim 1, wherein the control sample
is from non-involved tissue of the subject and the
substantial difference is a factor of at least about 2.

- 163 -

7.   The method of claim 1, wherein the control sample
is from non-involved tissue of the subject and the
substantial difference is a factor of at least about 5.

8.   The method of claim 1, wherein the level of
expression of the marker in the sample is assessed by
detecting the presence in the sample of a protein
corresponding to the marker.

9.   The method of claim 8, wherein the presence of the
protein is detected using a reagent which specifically
binds with the protein.

10.   The method of claim 9, wherein the reagent
comprises an antibody or fragments thereof.

11.   The method of claim 1, wherein the marker is
selected from the markers listed in Table 9.

12.   The method of claim 1, wherein the marker is
selected from the markers listed in Table 10.

13.   The method of claim 1, wherein the level of
expression of the marker in the sample is assessed by
detecting the presence in the sample of a transcribed
polynucleotide or portion thereof, wherein the
transcribed polynucleotide comprises the marker.

14.   The method of claim 13, wherein the transcribed
polynucleotide is a mRNA.

15.   The method of claim 13, wherein the transcribed
polynucleotide is a cDNA.

- 164 -

16. The method of claim 1, wherein the level of expression of the marker in the sample is assessed by detecting the presence in the sample of a transcribed polynucleotide or a portion thereof which hybridizes with a labeled probe under stringent conditions, wherein the transcribed polynucleotide comprises the marker.

17. A method of diagnosing a subject with multiple sclerosis, the method comprising the step of comparing:

a) a level of expression in a sample of the subject of each of a panel of markers independently selected from the markers listed in Tables 7-10, and

b) a normal level of expression of the panel of markers obtained from a control sample,

wherein the level of expression of the panel of markers is substantially different, relative to the corresponding normal level of expression of the panel of markers, indicates that the subject is afflicted with multiple sclerosis.

18. The method of claim 17, wherein the panel of markers comprises at least 5 markers.

19. The method of claim 17, wherein the control sample is from a non-diseased subject.

20. The method of claim 17, wherein the control sample is from non-involved tissue of the subject.

21. The method of claim 17, wherein the panel of markers comprises markers listed in Table 10.

22.   A method for monitoring the progression of
multiple sclerosis in a subject, the method comprising
the steps of:

a) detecting in a subject sample at a first point
in time, a level of expression of at least one marker,
wherein the at least one marker is selected from the
group consisting of markers listed in Tables 7-10;

b) repeating step a) at a subsequent point in time
with the at least one marker;

c) detecting a substantial difference between the
levels of expression detected in steps a) and b);

wherein the substantial difference between the
levels of expression indicates that the subject has
progressed to a different stage of multiple sclerosis.

23.   The method of claim 22, wherein at least 5 markers
are selected from the group of markers listed in Tables
7-10.

24.   The method of claim 22, wherein the at least one
marker corresponds to a transcribed polynucleotide or
portion thereof.

25.   The method of claim 24, wherein the samples are
collected from brain tissue.

26.   The method of claim 24, wherein the samples are
peripheral blood mononuclear cells.

27.   A method of assessing the efficacy of a test
compound for inhibiting multiple sclerosis in a
subject, the method comprising the step of comparing:

a) expression of a marker in a first sample
obtained from the subject and exposed to the test

compound, wherein the marker is selected from the group
consisting of markers listed in Tables 7-10, and

    b) expression of the same marker in a second
sample obtained from the subject, wherein the second
sample is not exposed to the test compound,

    wherein a substantially different level of
expression of the marker in the first sample, relative
to the second sample, is an indication that the test
compound is efficacious for inhibiting multiple
sclerosis in the subject.

28. The method of claim 27, wherein the first and
second samples are portions of a single sample obtained
from the subject.

29. The method of claim 28, wherein the level of
expression in the first sample approximates the level
of expression in a control sample.

30. A method of assessing the efficacy of a therapy
for inhibiting multiple sclerosis in a subject, the
method comprising the steps of comparing:

    a) expression of a marker in a first sample
obtained from the subject prior to providing at least a
portion of the therapy to the subject, wherein the
marker is selected from the group consisting of markers
listed in Tables 7-10, and

    b) expression of the marker in a second sample
following provision of the portion of the therapy,

    wherein a substantially different level of
expression of the marker in the second sample, relative
to the first sample, is an indication that the test
compound is efficacious for inhibiting multiple
sclerosis in the subject.

- 167 -

31. The method of claim 30,. wherein a substantially similar level of expression in the second sample, relative to the control sample, is an additional indication that the test compound is efficacious for inhibiting multiple sclerosis.

32. The method of claim 31, further comparing expression of the marker in a control sample, wherein expression of the marker in the second sample is similar to expression of the marker in the control sample.

33. A method of screening test compounds for inhibitors of multiple sclerosis, the method comprising the steps of:

a) obtaining a sample comprising cells from the subject;

b) separately maintaining aliquots of the sample in the presence of a plurality of test compounds;

c) comparing the expression levels of a marker in each of the aliquots, wherein the marker is selected from the group consisting of markers listed in Tables 7-10; and

d) selecting one of the test compounds which induces a substantially different level of expression of the marker in the aliquot containing that test compound, relative to other test compounds.

34. The method of claim 33, wherein the substantially different level of expression is a substantially lower level of expression.

35. The method of claim 34, wherein the substantially different level of expression is a substantially enhanced level of expression.

- 168 -

36. A kit for diagnosing a subject with multiple sclerosis, the kit comprising reagents for assessing expression of a marker selected from the group consisting of markers listed in Tables 7-10.

37. A kit for diagnosing multiple sclerosis in a subject, the kit comprising a nucleic acid probe wherein the probe specifically binds with a transcribed polynucleotide corresponding to a marker selected from the group consisting of markers listed in Tables 7-10.

38. A kit for assessing the suitability of each of a plurality of compounds for inhibiting progression of multiple sclerosis in a subject, the kit comprising:
     a) the plurality. of compounds; and
     b) a reagent for assessing expression of a marker selected from the group consisting of markers listed in Tables 7-10.

39. A kit for diagnosing multiple sclerosis in a subject, the kit comprising an antibody, wherein the antibody specifically binds with a protein corresponding to a marker selected from the group consisting of markers .listed in Tables 7-10.

40. A method of modulating a level of expression of a marker selected from the markers listed in Tables 7-10, the method comprising providing to diseased cells of a subject an antisense oligonucleotide complementary to a polynucleotide corresponding to the marker.

41. A method of modulating a level of expression of a marker selected from the markers listed in Tables 7-10, the method comprising providing to diseased cells of a subject a protein.

- 169 -

42. The method of claim 41, wherein the protein is
provided to the cells by providing a vector comprising
a polynucleotide encoding the protein to the cells.

43. A method of modulating a level of expression of a
marker selected from the markers listed in Tables 7-10,
the method comprising providing to diseased cells of a
subject an antibody.

44. The method according to claim 43, wherein the
method further comprises a therapeutic moiety
conjugated to the antibody.

45. A method of localizing a therapeutic moiety to
diseased tissue comprising exposing the tissue to an
antibody which is specific to a protein encoded from a
marker listed in Tables 7-10.

46. A method of screening for a test compound capable
of modulating the activity of a protein encoded from a
marker listed in Tables 7-10, the method comprising
combining the protein and the test compound, and
determining the effect of the test compound on the
therapeutic efficacy of the protein.

47. A method of screening for a bioactive agent
capable of interfering with the binding of a protein
encoded from a marker listed in Tables 7-10 and an
antibody which binds to the protein, the method
comprising:
        a) combining the protein, a bioactive agent and an
antibody which binds to the protein; and
        b) determining the binding of the protein or
fragment thereof and the antibody.

48. An antibody which specifically binds to a protein encoded from a marker listed in Tables 7-10.

49. The antibody of claim 48, wherein the antibody is a monoclonal antibody.

50. The antibody of claim 49, wherein the antibody is a humanized antibody.

51. A peptide encoded from markers listed in Tables 7-10.

52. A composition comprising the peptide of claim 51.

53. A composition capable of modulating an immune response in a subject, the composition comprising a protein encoded from a marker listed in Tables 7-10, and a pharmaceutically acceptable carrier.

54. A biochip comprising a panel of markers selected from the group of markers listed in Tables 7-10.

55. The biochip of claim 54, wherein the markers are selected for subjects suspected of having secondary progressive multiple sclerosis.

56. The biochip of claim 54, wherein the markers are selected for subjects suspected of having primary progressive multiple sclerosis.

57. The biochip of claim 54, wherein the markers are selected for subjects suspected of having relaxing-remitting multiple sclerosis.

58. The biochip of claim 54, wherein the markers are selected for subjects suspected of having multiple sclerosis, wherein the subjects are from a high-risk geographic region.
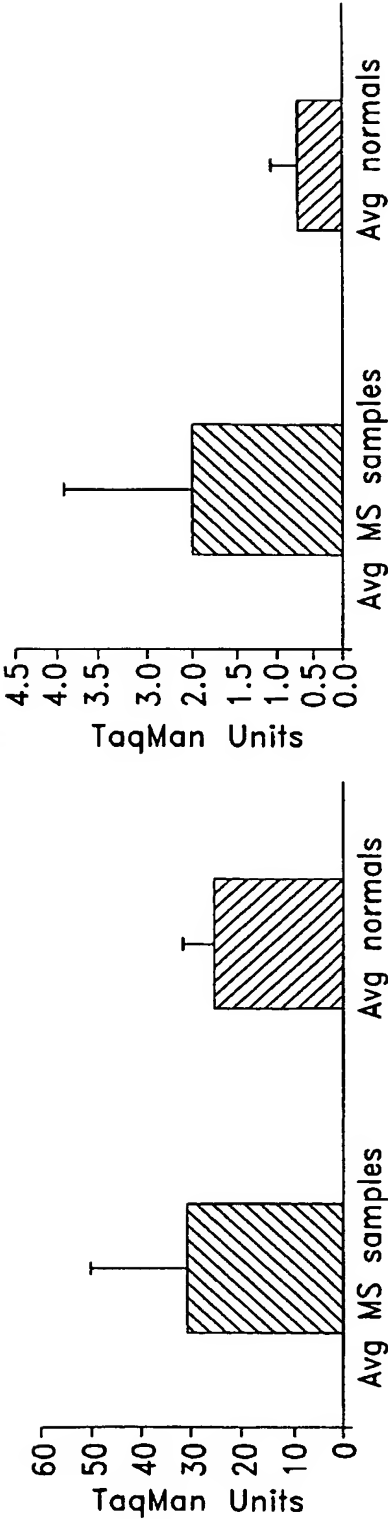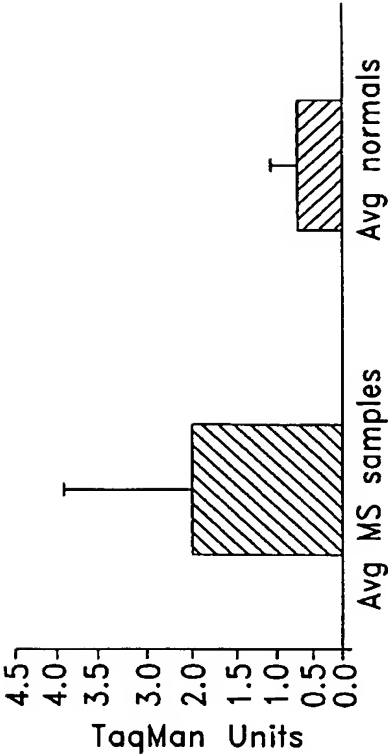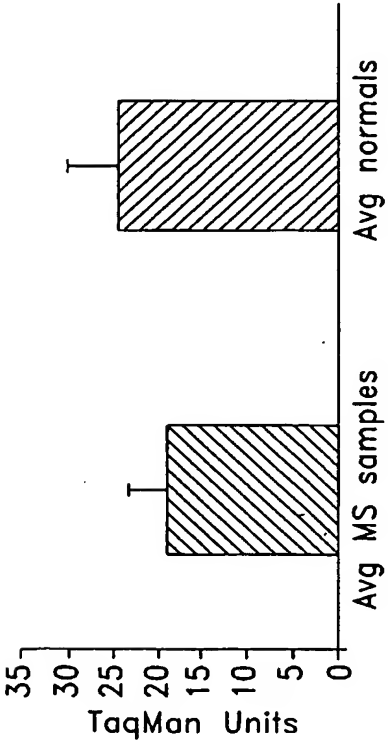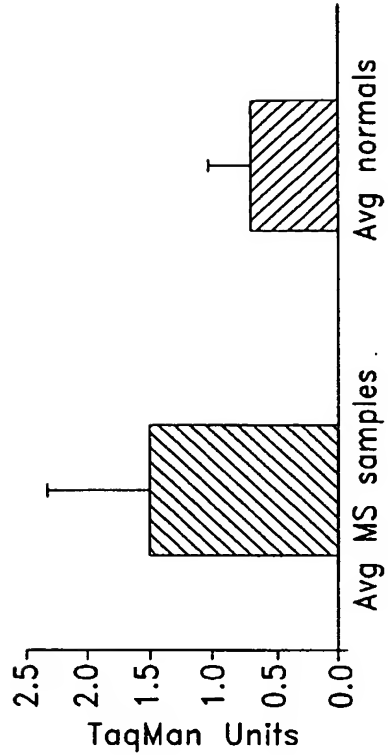
FIG. 1A

FIG. 1B

FIG. 1C

FIG. 1D
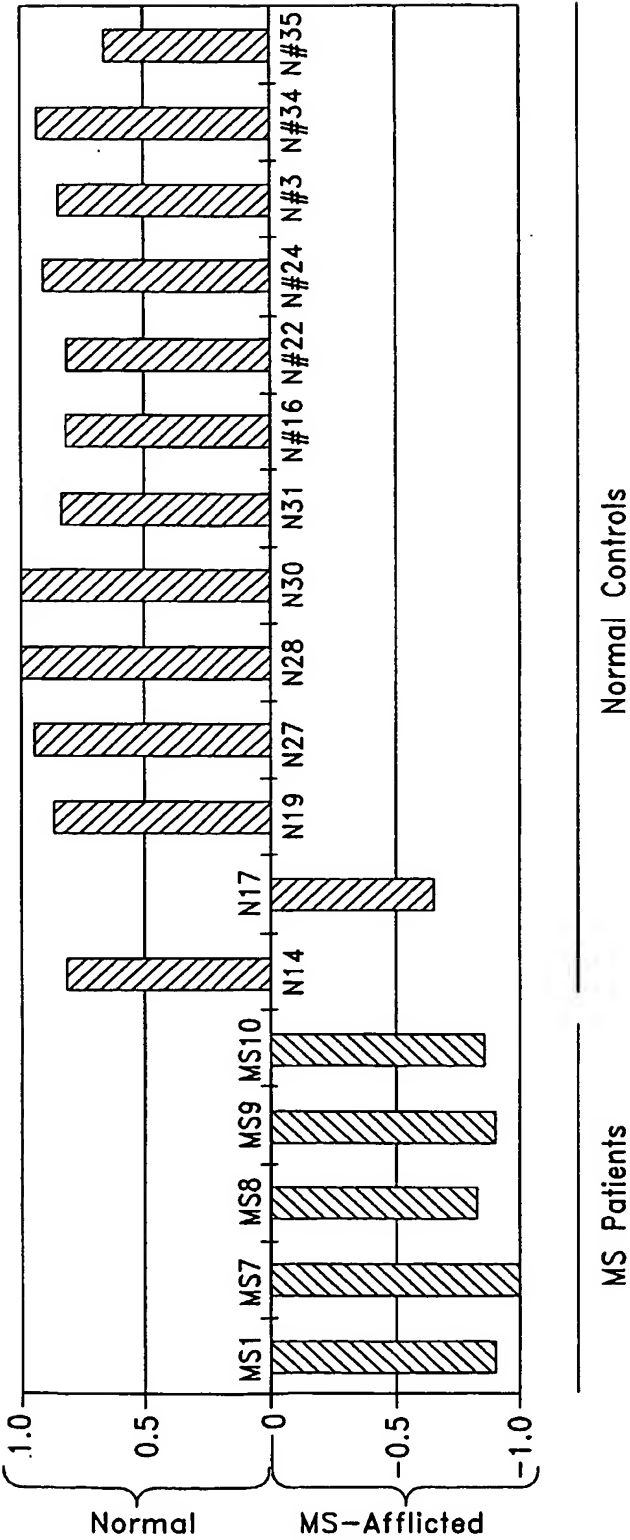
FIG. 2

INTERNATIONAL SEARCH REPORT

	International application No.
	PCT/US02/09305

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C07H 21/04; C12Q 1/68
US CL : 435/6; 536/23.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/6; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	VAN BOXEL-DEZAIRE et al. "Decreased interleukin-10 and increased interleukin-12p40 mRNA are associated with disease activity and characterize different disease stages in multiple sclerosis" Annals of Neurology, June 1999, Vol. 45, No. 6, pages 695-703, see entire document.	1-26
A	SEDLACEK et al. "Evolutionary conservation and genomic organization of XAP-4, an Xq28 located gene coding for a human rab GDP-dissociation inhibitor (GDI)". Mammalian Genome. October 1994, Vol. 5, No. 10, pages 633-639, see entire document.	1-26

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier application or patent published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 July 2002 (23.07.2002)	**2 2 AUG 2002**
Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Jeanine Enewold Goldberg
Facsimile No. (703)305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/09305

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-26, in part, namely gene XAP4

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1)) (July 1998)

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

Group 1-808, claim(s) 1-26, in part, drawn to methods of diagnosing a subject with multiple sclerosis by comparing expression of a marker listed in Tables 7-10 to normal expression levels or using a panel of markers for expression analysis of multiple sclerosis. It is noted that Groups 1-808 correspond to the 808 markers of Tables 7-10. Therefore, the first mentioned invention is the methods of claim 1 to the extent that they apply to expression analysis of XAP4 (Accession U67322) and panels of at least five markers wherein the panel comprises XAP4. **Group 1, the first mentioned invention, is the invention which will be searched in accordance with PCT Article 17(3)(a).** Additional groups may be elected. For example, if Group 2 is elected, and the proper fees are paid, then claim 1 will be examined to the extent that they apply to methods of expression analysis with respect to OA48-18 (Accession Number AF069250) or a panel of five makers comprising said marker recited in claim 1. Upon election of an invention to be searched in addition to group 1, please identify the number of the gene to be searched in the method claims.

Group 809-1616, claim(s) 27-35, in part, drawn to methods of assessing the efficacy of therapy or compounds by detecting expression of a marker listed in Tables 7-10.

Group 1617-2424, claim(s) 36-38, 54-58 in part, drawn to kits comprising nucleic acids of Tables 7-10 and biochips.

Group 2425-3232, claim(s) 36, 39, 48-50 in part, drawn to kits comprising antibodies which binds with a protein corresponding to the markers of Tables 7-10.

Group 3233-4040, claim(s) 40, drawn to methods of modulating expression using antisense oligonucleotides .

Group 4041-4848, claim(s) 41-42, drawn to methods of modulating expression using a protein of Tables 7-10.

Group 4849-5656, claim(s) 43-44, drawn to methods of modulating expression using an antibody of Tables 7-10.

Group 5657-6464, claim(s) 45, drawn to methods of localizing a therapeutic moiety.

Group 6465-7272, claim(s) 46-47, drawn to methods of screening for test compounds by monitoring expression of protein of Tables 7-10.

Group 7273-8080, claim(s) 51-53, drawn to peptides encoded from markers listed in Tables 7-10.

The inventions listed as Groups 1-89 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons.

The claims are drawn to detecting expression of numerous genes, in the alternative, as diagnostic of multiple sclerosis. It is well known in the art that various genes are differentially expressed in multiple sclerosis cells such that one may detect a predisposition of MS based upon overexpression of a gene compared to the normal expression level of the gene. The disclosure specifically states that "A study by Whitney and colleagues utilized genetic expression data from one patient to compare MS tissue to non-MS tissue, revealing that about sixty-two genes were differentially expressed, including the Duffy chemokine receptor, interferon regulatory factor-2 and tumor necrosis factor alpha receptor 2." (page 3, lines 5-15). Moreover, it is noted that the art teaches the association of over expression of many genes in MS (Baranzini, J. Immunology Vol 165, pages 6576-6582, 2000). Therefore, there is no special technical feature which links the claims as defined by PCT Rule 13.2, and therefore lack of unity is present between the groups.

Additionally, methods which are comparing expression of one gene are not the same as method comparing expression of a different gene. The expression level of one gene as indicative of MS does not bear any indication of how a different gene will be expressed in cancer cells. The molecules, namely genes, which are relied upon in each of the methods do not share a common structure.

With respect to the markers, namely the polynucleotides and polypepetides, each of the genes provided in Table 7-10 are identified by Accession Number indicating that the sequence of the genes were known in the art. For example, XAP4, Genbank Accession Number U67322, available in 1999 contains both a nucleic acid and a polypepetide sequence. Therefore, the genes themselves are not contributions over the art as exemplified in the Tables themselves.

The groups comprising polynucleotides, kits, polypeptides, antibodies are additionally drawn to multiple, distinct products lacking the same or corresponding special technical features. The nucleic acids are composed of nucleotides and function in, e.g., methods of nucleic acid hybridization or amplification. These groups are directed to different combinations of nucleic acids which are different from one another and may be employed in different methods. The polypeptides differ in both structure and function from either the nucleic acids or the transgenic organisms. The polypeptides are composed of amino acids linked by peptide bonds and arranged in a complex combination of alpha helices, beta pleated sheets, hydrophobic and hydrophilic domains. The polypeptides also differ in function, e.g., fusion proteins with an enzymatic functions. The antibodies are composed of amino acids linked by peptide bonds, antibodies are glycosylated and their tertiary structure is unique, where four subunits (2 light chains and 2 heavy chains) associated via disulfide bonds into a Y-shaped symmetric dimer. The antibodies function in immunoassays. As products of different sets of Groups differ from each other in structure, function, and effect, they do not belong to a recognized class of chemical compound, or have both a "common property or activity" and a common structure as would be required to show that the inventions are "of a similar nature".

The methods are similarly not the same because they rely upon different products and have different objectives, reagents and results. Therefore, the methods lack unity.

Continuation of B. FIELDS SEARCHED Item 3:
file medline biosis caplus embase scisearch
xap4, xap 4, xap-4 or u67322, hbv associated factor